DESIGN AND ON-LINE COMPUTER CONTROL OF FERMENTOR

A Thesis Submitted
In Partial Fulfilment of the Requirements
for the Degree of
MASTER OF TECHNOLOGY

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by
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To

The Memory of My

brother

Who Gave so Much

And Asked so Little

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CERTIFICATE

It is certified that this work on 'DESIGN AND ON-LINE COMPUTER CONTROL OF FERMENTOR' has been carried out under my supervision and that this has not been submitted elsewhere for a degree.

Date: May 14, 1976

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This thesis has been approved for the award of the Degree of Master of Technology (M.Tech.) in accordance with the regulations of the Indian Institute of Technology Kanpur
Dated. 15.5.76 24

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Author

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ABSTRACT

The contents of this thesis present the complete design and fabrication of a 14-litre capacity fermentor which is provided with sensing probes for pH and dissolved oxygen concentration. Further a thermocouple is used for temperature measurements. This fermentor is coupled to IBM 1800 for the on-line control of its temperature and pH during Baker's yeast fermentation. This work also involves the design of an active low pass noise filt and a valve driver. This basically represents the control of two most important controlling variables during fermentation and thus becomes a part of work towards the use of digital computer for process control in biochemical industry. The programs are also written to service the interrupt, from process terminals, process data acquisition and control.

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CHAPTER 1

INTRODUCTION

The digital computers have been successfully employed as data processing aids in business and management for a long time. The design of the computers, which is of use for on-line control of industrial processes and for other real time applications was suggested in early sixties. Enough experimentation was done to develop the capability of computer to operate in real-time and with speed and accuracy to control critical processes with reliability and safety.

With the increasing use of computers in the petroleum and chemical industries, endeavours were also made and are being made these days to use the high data processing capability of computers to create the optimum environment for a desired fermentation performance. In addition, computer control increases process output, raises efficiency, improves product quality and cuts down operating cost.

Till early 1950s, a fermentor had almost no controls. It was simply an agitated vessel with all variables monitored and controlled by an operator. However in the last decade more and more attention was paid towards the automatic control of isolated factors e.g. temperature, pH and dissolved oxygen concentration etc. With the time a need was felt to develop

suitable sensors which may cope with the speed and accuracy of 'Real-time computers', which ultimately brought this technology of on-line computer control to biochemical industry and result was that by 1970 there were many laboratory fermentors in U.S.A., Japan and France which were partially or completely controlled by computers. However, because of the rather involved nature of the biochemical processes compared to the usual chemical processes, the application of computers in biochemical processes is progressing at a slower rate than in chemical and other industries. This is not due to the shortage of computer hardware but rather due to lack of complete knowledge about the metabolic path ways, regulatory mechanisms and the ways in which the environmental factors affect the fermentation.

The present status of computer applications in the field of biochemical engineering is that various optimization models have already been developed and were found quite successful but still the efforts are going on to develop extremely sophisticated sensors for getting more information regarding metabolic activities, and to study the interactive nature of different variables to attain dynamic optimization for fermentation control.

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CHAPTER 2

LITERATURE REVIEW

2.1 Direct Application of Computers in Fermentation Processes:

The idea of using computers to control fermentation was first proposed by Fuld (1960)[1]. Since then there has been significant developments resulting in the application of various sensors and different type of computers to monitor fermentation processes. Recently new ideas about computer control techniques have been disseminated and all these efforts have the same goal of establishing optimum environmental conditions.

Grayson (1969)[2] operated 36 fermentors for batch production of penicillin under Direct Digital Control (DDC). The computer was used to control the temperature, pH, air flow rate and the addition of antifoam.

Yamashita and his coworkers (1969)[3] described a concept of application of computers for control of large-scale glutamic acid fermentations. The plant was first manipulated with a relay-type sequence control system (Mori and Yamashita, 1967)[4]. A digital computer was then developed to replace the logic circuit of the sequence control system. Thereafter, Hoshi et al. (1967)[5] and Yamashita (1967)[6] employed digital computers for controlling several variables, first at pilot plant level and then in large scale units.

Henry (1970)[7] got success in replacing the conventional analog controllers to accomplish complex control performance. The computer monitored the following control variables: temperature of the culture broth, vessel pressure, pH of the broth, air flow rate, and addition of the antifoam agent.

Nyiri, Humphrey, Jefferis have done a lot of work in controlling fermentation units with on-line computers, during last 5 years. In fact all the work done in early seventies until today is oriented towards data acquisition using the best available sensors, data analysis, development of computer programs for on-line control purpose, application of mathematical models and heuristic methods to resolve the numerical and logical problems of cell growth, mutation and product formation. Finally, all this information will help in developing algorithms for on-line optimization, prediction techniques and dynamic optimization.

Traditionally speaking, process control in the fermentation industry has meant control of purely physical and physicochemical variables such as temperature, agitation speed, sparge rate, vessel pressure and pH etc. In some cases, computers have been used to perform the above functions, which had hitherto been handled by analog and digital controllers. (Grayson, 1969; Yamashita et al. 1969; Geranton, 1973). This approach of using the computer as a direct replacement for

analog system elements is however inadequate because it does not take in to account the significantly interactive nature of fermentation process variables.

Most recent work and achievements in this area of computer control of fermentor are as follows:

Burns (1971)[8] reported a device for direct measurement of antibiotic inhibition zones on agar plates. An on-line operating IBM 1800 computer monitors the function of this device. Tuffile and Pinho (1970)[9] used an IBM 1130 computer and a suitable program to study the changes of viscosity values during streptomycin fermentation.

Nyiri (1971)[10] made a data analysis oriented application of the computer to the fermentation processes, which provides the opportunity to determine the actual physico-chemical status of the culture broth and offers insight into some of the physico-chemical and biochemical conditions of the cells. By this means the effect of control variables on cell growth and metabolism can be studied on a real time basis.

Geranton (1973)[11] worked for developing the design and construction of system software involved in on-line control of fermentors. Efforts were made to give a generalized method for writing control programs for controlling biochemical reactors.

Humphrey et al. (1973)[12] developed a control model for optimization of batch fermentation processes through on-line computer analysis and control. Considering choke out problems of batch fermentors due to limited oxygen uptake rate and for attaining optimal control, the control was so oriented as to achieve a rapid high cell density, to control the carbon substrate feed for the maximum product to growth ratio and to maintain the dissolved-oxygen levels above choke out conditions by repeated draw-off of cell bio-mass with broth make up. Equations relating these factors were developed, and optimized on-line control was achieved.

Furthermore, Jefferis (1973)[13] worked on control of oxygen uptake rate of growing culture of saccharomyces cerevisiac in a 14-litre fermentor coupled to a PDP-11 computer. This work also gives a more complex data structure for the computer control, modelling and optimization of fermentation processes, with a little discussion on the computer hardware part. Lateron, Bourdaud and Foulard [14] carried out experimental work on the optimal control of yeast batch fermentation processes. They developed model for the cell growth and optimal control strategy.

Lane (1973)[15] discussed the problems involved in measurement of biomass concentration on a real time basis and developed an on-line turbidometric method for measuring the bio-mass concentration using a process computer. Ake Unden

and Heden (1973)[16] worked in the area of on-line continuous culture cultivation and optimization and gave a computer controlled continuous culture attack at the physiological level and used a Hill climbing optimization approach.

Humphrey et al. (1972)[17] used a highly instrumented computer-coupled fermentor for collection of physical and chemical environmental data for continuous and instantaneous system analysis and then used this information for the feed-back control of the fermentation at its optimal operating point. Nyiri et al. (1974)[18] developed,for pilot scale application a programmed data acquisition and data analysis system which makes it possible to obtain instantaneous information on the rheological, physiological and bio-chemical conditions of the process. A biological interactive control program was also developed, which produced appropriate control actions and process trends based upon fundamental relationships between the environment and the living cells.

2.2 Baker's Yeast Fermentation

The baking of bread has been practised for centuries. Egyptians, Jewish, Greek and Roman histories reveal many interesting facts concerning the baker's yeast fermentation and brewing etc. Krunitz (1775) wrote an encyclopedia on yeast. Mason (1792) prepared the compressed yeast for the first time. Thereafter in early 19th century Dutch people

and Hollanders got success in making compressed yeast. Landersdorff in early 19th century developed methods for souring the wort, improved methods for selecting stock yeast and introduced some controls in fermentation industry. He also suggested the use of acid in fermentation to prevent contamination and stimulate growth.

Mautner (1846), developed 'The Vienna Process', to prepare baker's yeast on a commercial scale. Yields of 10 to 14 per cent on the basis of the grain were obtained with approximately 30 per cent ethyl alcohol formation. Soon after, 'Air Process' was developed by Eusebius Bruun in 1877, giving about 20 per cent commercial yeast and 20 per cent alcohol from grain.

Processes based on three patents were introduced universally in the years 1920-25. They produced yeast from grain molasses in batch fermentation. Yeast yields of approximately 100 per cent based on molasses weight, were common. Hayduck (1915) investigated the utilization of molasses and ammonia as a source of 'C' and 'N' for yeast propagation and he gave a commercial process known as 'Hayduck Process'. Yields as high as 200 per cent based on the amount of sugar in the wort have been obtained by this process.

Thereafter, 'Continuous Fermentation' was developed by the Germans in 1919, which offered possibilities for the continuous addition of nutrients and the continuous withdrawl

of the yeast from the fermentor.

Menzisky (1950) [19], has done a lot of work in yeast fermentation. He reported that the molasses 'with a lower sugar content and a higher organic nitrogen content give a better yield than a material with a higher sugar content and lower nitrogen content'. He also found that the yield of yeast from molasses was highly dependent upon biotin content of the medium.

de Becze and Liebmann (1944)[20] reported, that adequate aeration is one of the most important factors in yeast production.

White and Munns (1950)[21] found that the Baker's yeast also required certain vitamins for better yields.

Menzisky (1950) found that varying the pH between the range of 4 to 6 had no influence on the yield. However, low pH was helpful in restricting the development of many bacterial species.

Merritt (1957) [22] gave the idea of producing active dry yeast and he found that this active dry yeast was uniform and stable over required periods of time, was convenient to weigh, rehydrated readily and was economical. This was the time when Baker's yeast industry was almost fully developed and not much work was done in this area in 1960's and later.

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CHAPTER 3

EXPERIMENTAL

The complete experimental set-up consists of

(1) Physical layout of the system

(2) Fermentation reaction

(3) Control scheme

3.1 Physical Layout of the System:

A schematic representation of the fermentor and control scheme has been shown in Figure 1. It consists of a 14 litre capacity glass fermentor, in which Baker's yeast fermentation is carried out. The fermentor is provided with a thermocouple for measuring temperature of the system and a pH-probe for measuring pH of the broth.

In addition, system consists of an air filtering unit, air compressor, a constant temperature cooling bath, two recorders for recording temperature and pH simultaneously. Though, the signal amplification, noise filtering unit and valve driving units are also part of the system but they have been discussed in detail in Chapter 5.

Photograph (1) shows an overall view of the experimental set-up.

3.2 Fermentation Reaction:

Baker's yeast fermentation is used as the system reaction. The complete process involves two steps:

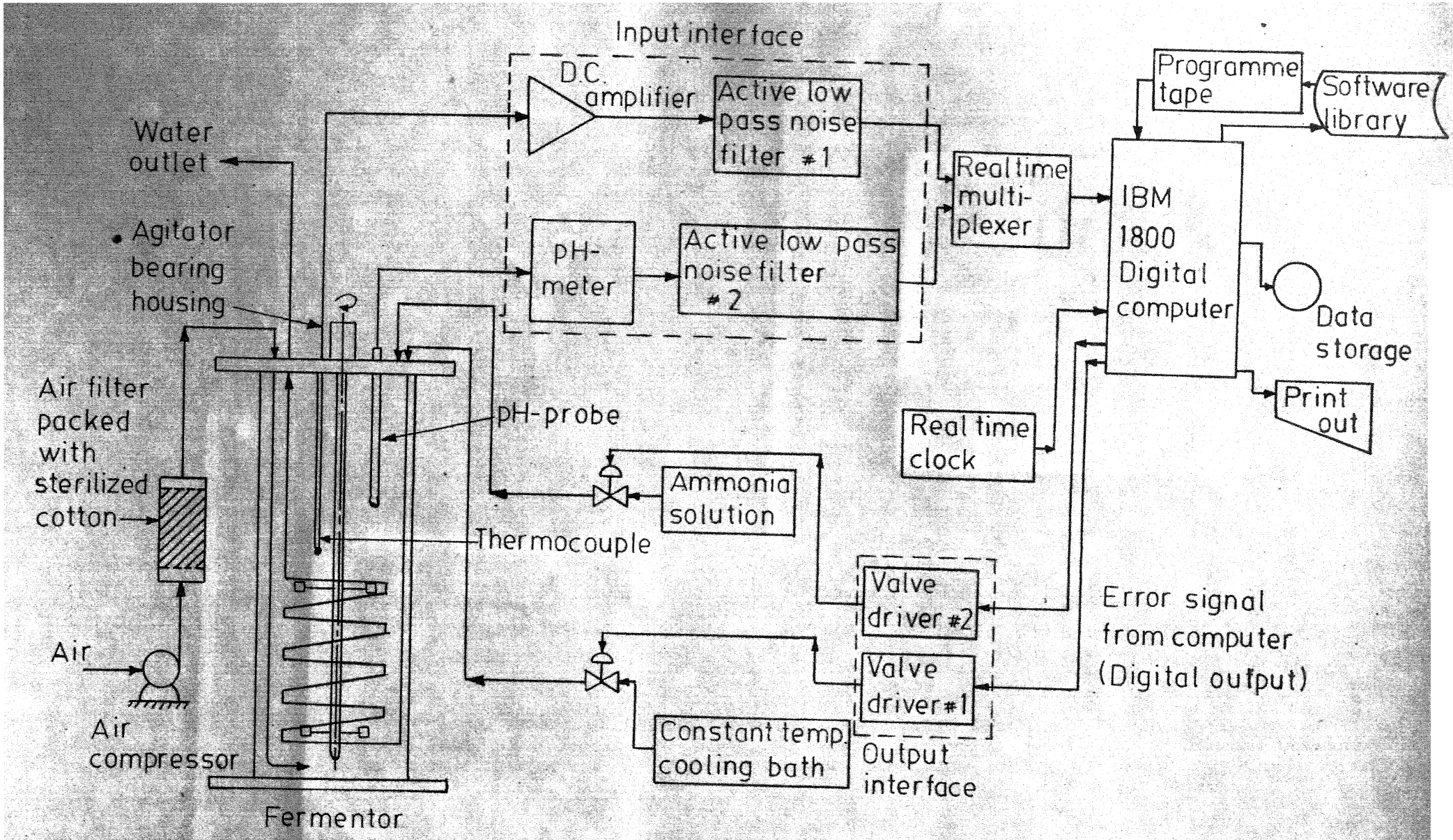
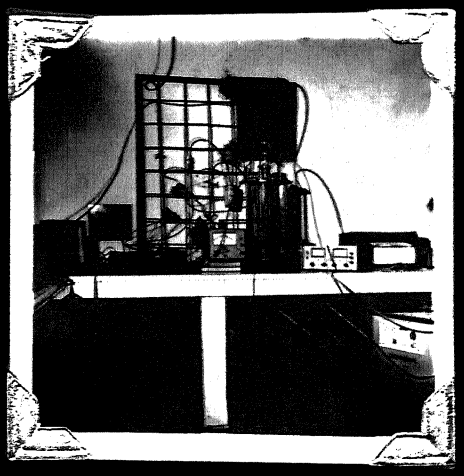
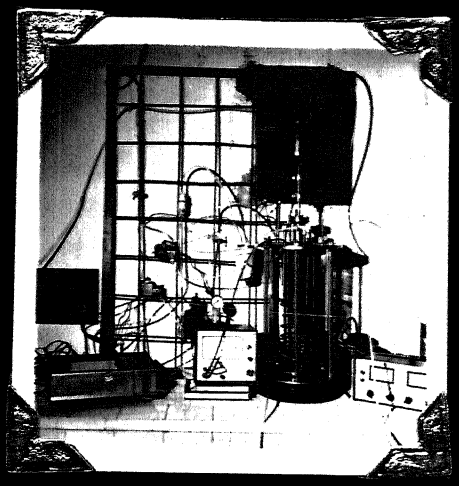


Fig. 1 - Physical layout of the system.



PHOTOGRAPH 1: EXPERIMENTAL SET-UP



PHOTOGRAPH 2: FERMENTOR WITH AUXILIARIES

(a) Preparation of salt medium

Chemicals: Volume of liquid in the fermentor = 10 litres

Crude cane molasses (4o beet solution)	= 300 gms
Calcium dibasic phosphate	= 2 gm
Urea	= 4 gm.
Ammonium Mono-Hydrogen phosphate	= 2 gms
Concentrated sulfuric acid	= 4 cc
Phosphoric acid	= 0.5 cc

The molasses, calcium dibasic phosphate and sulfuric acid are dissolved together and autoclaved in pyrex bottle and then the mixture is cooled. The sterilized urea, ammonium mono-hydrogen phosphate and phosphoric acid are further added to it. Now the distilled water is added to make the total volume 2 litres and finally the pH of the solution is adjusted to 4.8.

(b) Yeast growth and fermentation:

First, the fermentor vessel along with all sensors, agitator etc. are sterilized using steam or rinsing with ethyl alcohol.

To start with fermentation, 400 gms of fresh Baker's yeast are suspended in about 8 litres of distilled water, and are introduced into the fermentor vessel.

The medium is now introduced into the fermentor and then agitation as well as aeration are started. The growing

yeast requires a strong aeration, which is done by using a small compressor and air is introduced at a pressure of 5 psig. Just prior to the air inlet port, air is passed through sterilized cotton packed column, which avoids contamination due to aerating air.

As the reaction starts, small amount of heat is released and thus the fermentor temperature starts increasing. To control this temperature at an optimum value of 28°C, we cool the fermentor by circulating cooling water at a constant temperature of 20°C and thus temperature is controlled within the limits of ± 1°C.

Simultaneously the pH of the fermentor broth also starts falling gradually and thus base (ammonia solution 1 part and 3 parts water) is added to bring the pH to optimum value of 4.8, which is also controlled by actuating the base addition valve and it is controlled within limits of ± 0.1 pH unit.

Periodic samplings at an interval of 15 or 30 minutes are made from time to time and when the budding of the yeast was well under way, the rate of aeration is increased to a extent that there is a limited foam formation. Moreover, to control foam formation, castor oil is added from time to time.

During fermentation, the concentration of sugar is maintained at a low level in order to favour its utilization for the manufacture of yeast cells rather than for the production of ethyl alcohol [23].

Fermentation of Baker's yeast being an aerobic reaction, so the main by products of this reaction are CO_2, water, alcohols and esters. Reaction can be written as

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O \quad \text{(aerobic)}$$

In all, the reaction is almost complete in 8 hours of time.

(c) Washing of yeast and its preservation:

At the end of the fermentation, the medium is transferred to a cold room at $4^{\circ}C$ to allow the settling of the yeast [24]. After 20-30 hours the supernatant liquid is withdrawn and then settled yeast is washed with ethyl or propyl alcohol for about 1 hr. and finally it is dried at $60^{\circ}C$ for 20 hrs and then packed in tins.

Low temperatures are necessary for the storage of compressed yeast because molds and bacteria cause the cake to deteriorate rapidly at room temperature.

A method, which is used for estimating sugar concentration in a particular sample has been discussed in Appendix I. Also the method for estimating cell growth is given in Appendix II.

3.3 Control Scheme

The entire control scheme consists of

(1) Process (Fermentor)

(2) Measuring elements (Thermocouple and pH-probe)

(3) On-line computer (IBM 1800)

(4) Final control element (Solenoid valve)

Figure 2 represents a block diagram for the control scheme in general.

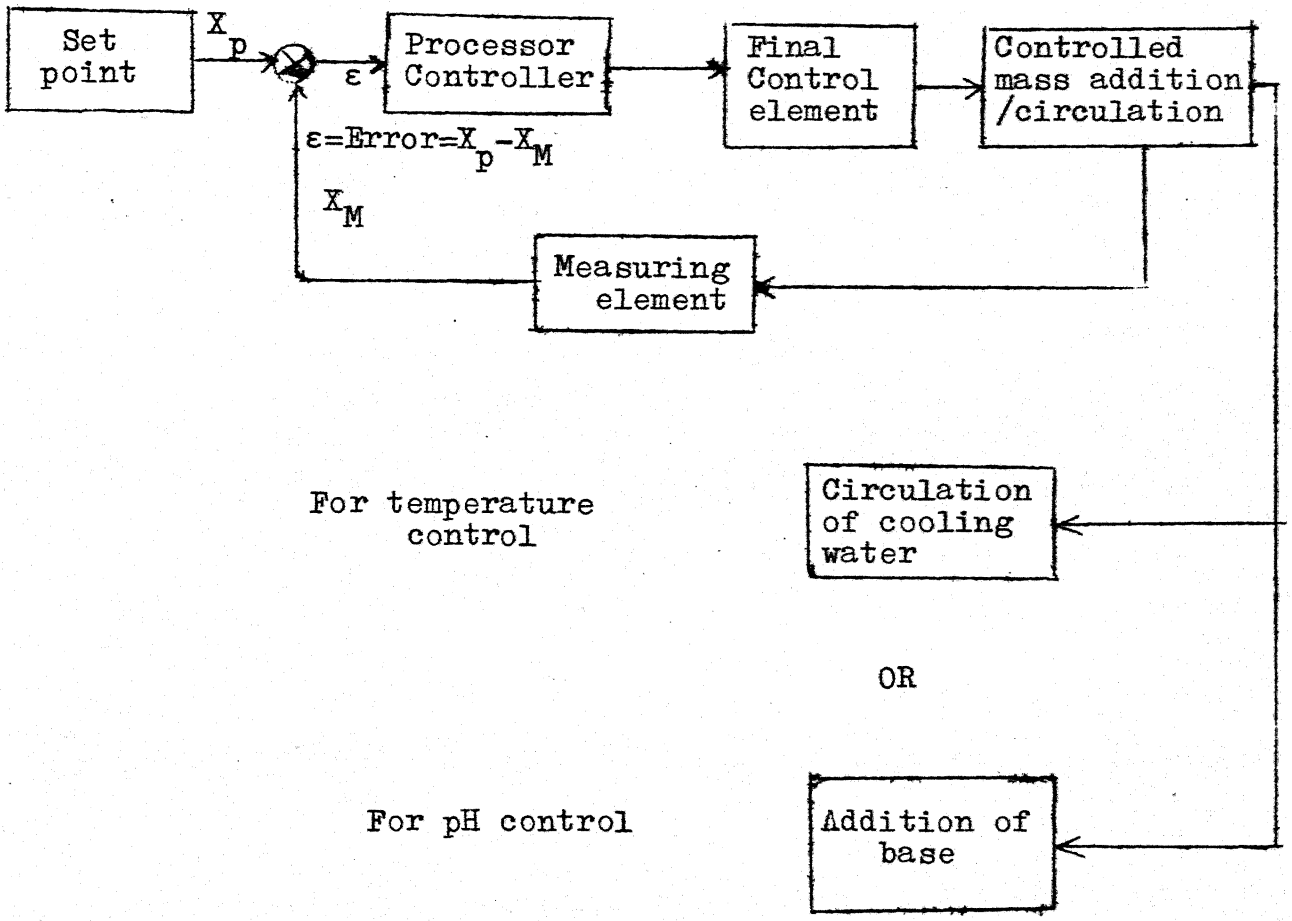


FIGURE 2: BLOCK DIAGRAM OF CONTROL SYSTEM

Here the control system is a closed loop system because the measured value of the controlled variable is fed to the computer. This value in turn is read and compared with a set value. If the difference between the set value and the measured value is more than or equal to the prescribed accuracy limit of that specific variable, then a digital output level is given by the computer, which actuates the valve driver and thus solenoid valve gets opened and remains open until the value of the variable in the process system reaches the set value (within accuracy limits) and then the next signal comes which closes the valve. Thus in this way temperature is controlled by circulating cold water in the cooling coil through a solenoid valve and pH, by addition of base through a solenoid valve.

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CHAPTER 4

CONSTRUCTIONAL DETAILS OF THE FERMENTOR

Our laboratory fermentor is a 14 litre capacity fermentor used for studying metabolic processes in submerged culture, under controlled conditions of temperature, pH, agitation, aeration. The assembly as shown in photograph (2) is large enough for realistic pilot plant studies, yet it is small enough to be placed in an autoclave, for thorough sterilization of the entire assembly. Provision has also been made to sterilize it by introducing steam under low pressures.

Problems of friction and freezing of the agitator shaft are eliminated by a specially designed steam-proof ball-bearing housing. Moreover, it incorporates a leak proof top closure, with leak proof seals and a silicone lubricant to prevent any leakage.

In addition, the design provides quick dismantling, easy removal and placement of the sensors in cleaning the entire assembly.

Thus with these design features, it is possible to eliminate completely the danger of contamination through the agitator housing, top cover and other probe seals.

Various constructional details of the fermentor and its auxiliaries are discussed below:

(1) Fermentation Chamber:

Our laboratory fermentor consists of a cylindrical glass vessel, approximately 18.0 inch high and 8.0 inch in diameter having an overall capacity of 14 litres. Fermentor top is made of a 1/2 inch thick stainless steel round plate 12½ inch in diameter. At the bottom of the cylinder, another supporting stainless steel plate equal in diameter to upper one and 1/4 inch thick is provided and both are tightened together with six stainless steel tie rods.

In the inner side of the top plate an O-ring groove equal in diameter to that of the fermentor top is made, so that the O-ring seal makes the fermentor leak proof. Moreover the glass vessel provides maximum visibility with ease of cleaning.

(2) Head Assembly:

The air line, acid/base addition line, air exit port, antifoam addition line, steam inlet, sampling port and inoculation port, all made of 1/2 inch diameter stainless steel pipe, are introduced through the head and are screwed in the top plate as shown in Figure 3. To prevent any leakage, teflon tape was used, while tightening these ports and the probe seals. In addition, all these ports are closed by stainless steel threaded caps at the top. Besides these, the head supports the agitator shaft, thermocouple well, cooling coil inlet and outlet, pH-probe and D-O_2 probe.

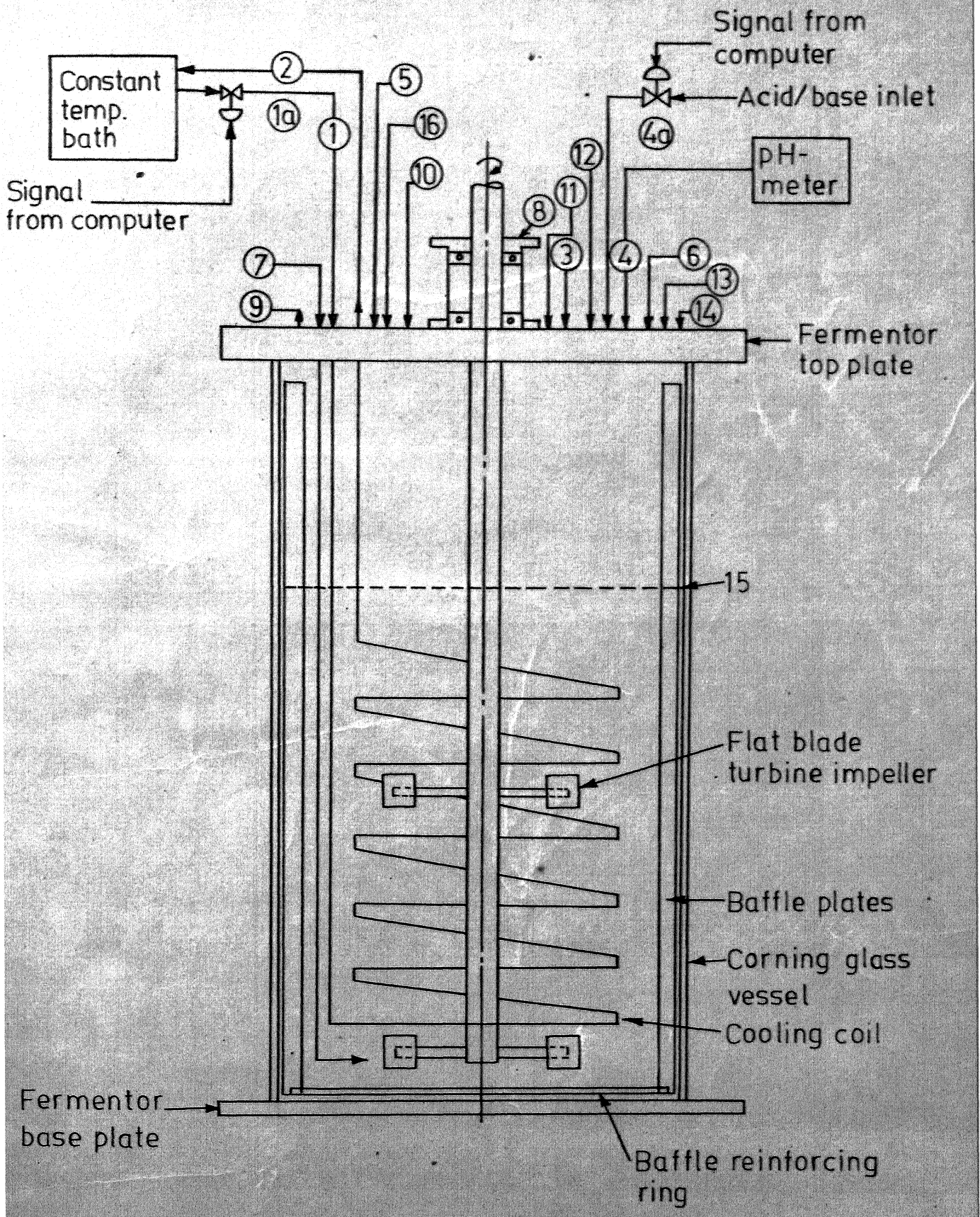


Fig.3 Typical fermentor with auxiliary equipment.

KEY TO FIGURE 3

1	Coolant Inlet
1a	Control Valve for Coolant
2	Coolant Outlet
3	Thermocouple Well and Thermo Couple which sends signal to Computer
4	pH-Probe and pH-meter which sends signal to Computer
4a	Acid/Base Flow Control Valve
5	Dissolved Oxygen Probe Connected to Recorder
6	Sampling Port
7	Sterile Air Inlet
8	Sealed Bearing Agitator Housing
9	Air Exhaust
10	Auxiliary Liquid Addition
11	Foam Probe
12	Anti Foam Addition
13	Inoculation Port
14	Substrate Feed
15	Liquid Level
16	Steam Inlet

Figures 4 and 5 show the probe seal fittings and design details for pH-probe and D-O_2 probe, respectively.

(3) Agitator:

The agitator shaft enters the fermentor through a specially designed leak proof ball bearing housing as shown in Figure 6, which consists of two sealed ball bearings, fixed within the housing, $2\frac{3}{4}$ inch apart. The space between the ball bearings is packed with teflon coated asbestos (PTFE) to prevent any air, liquid or steam leakage.

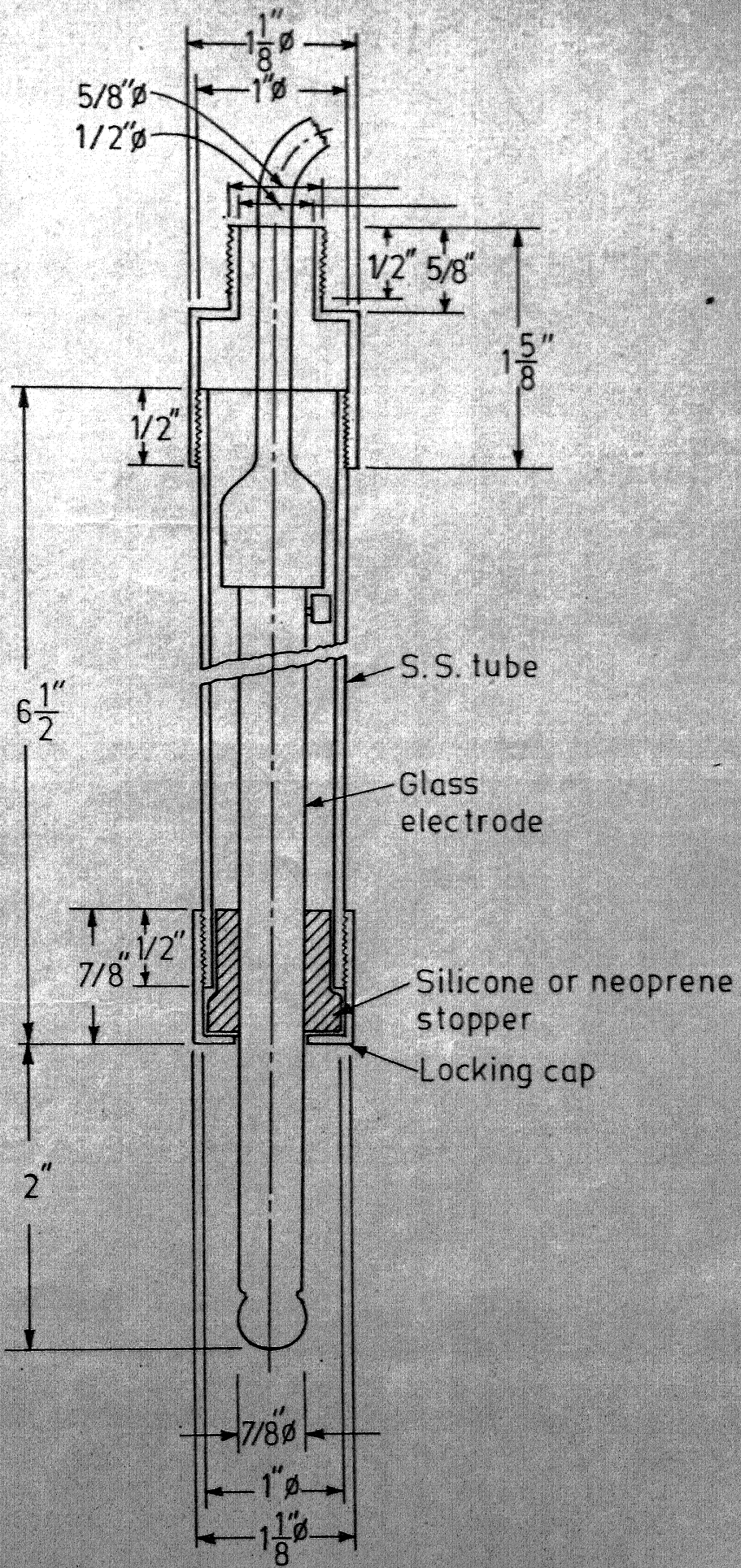
The agitator assembly consists of a 1/2 inch diameter stainless steel shaft, fitted with two, 4-bladed turbine type impellers, which are adjustable at any height and can be removed for cleaning. This system has been designed envisaging all design features to obtain a variable speed from 100-1000 rpm and driven by a 0-5 hp. D.C. motor.

(4) Baffles:

Four stainless steel baffles $17\frac{1}{2}$ inch length and 1 inch breadth are provided all along the fermentor height to prevent mass swirling and increasing turbulence. At the bottom of the jar they are welded to a reinforcing ring which makes them rigid and maintains uniform spacing.

(5) Air Sparger:

Sufficient amount of aeration is attained by introducing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Glass electrode
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Silicone or neoprene stopper
Locking cap
2"
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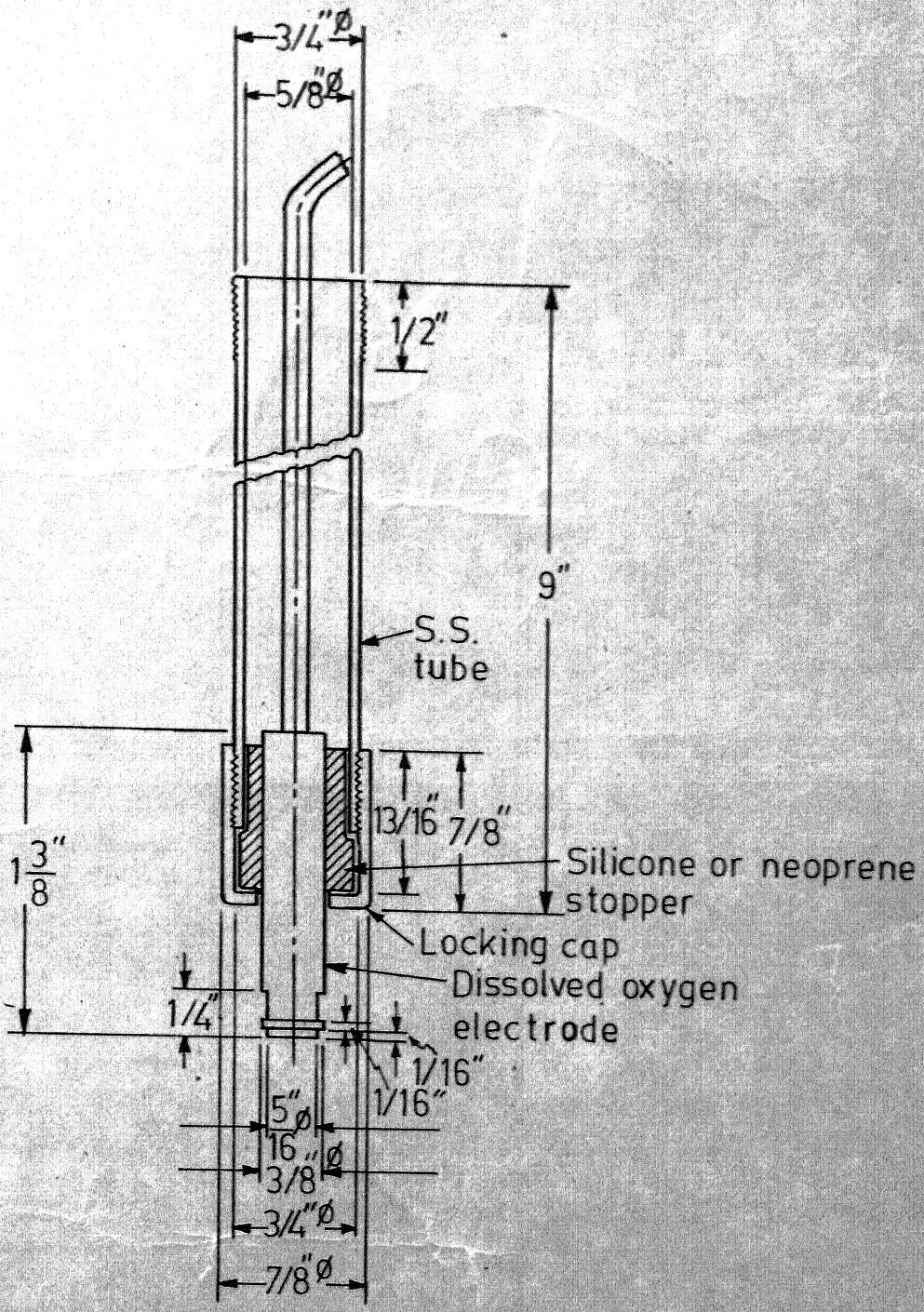

Fig. 5.- Seal for D-O_2 probe.

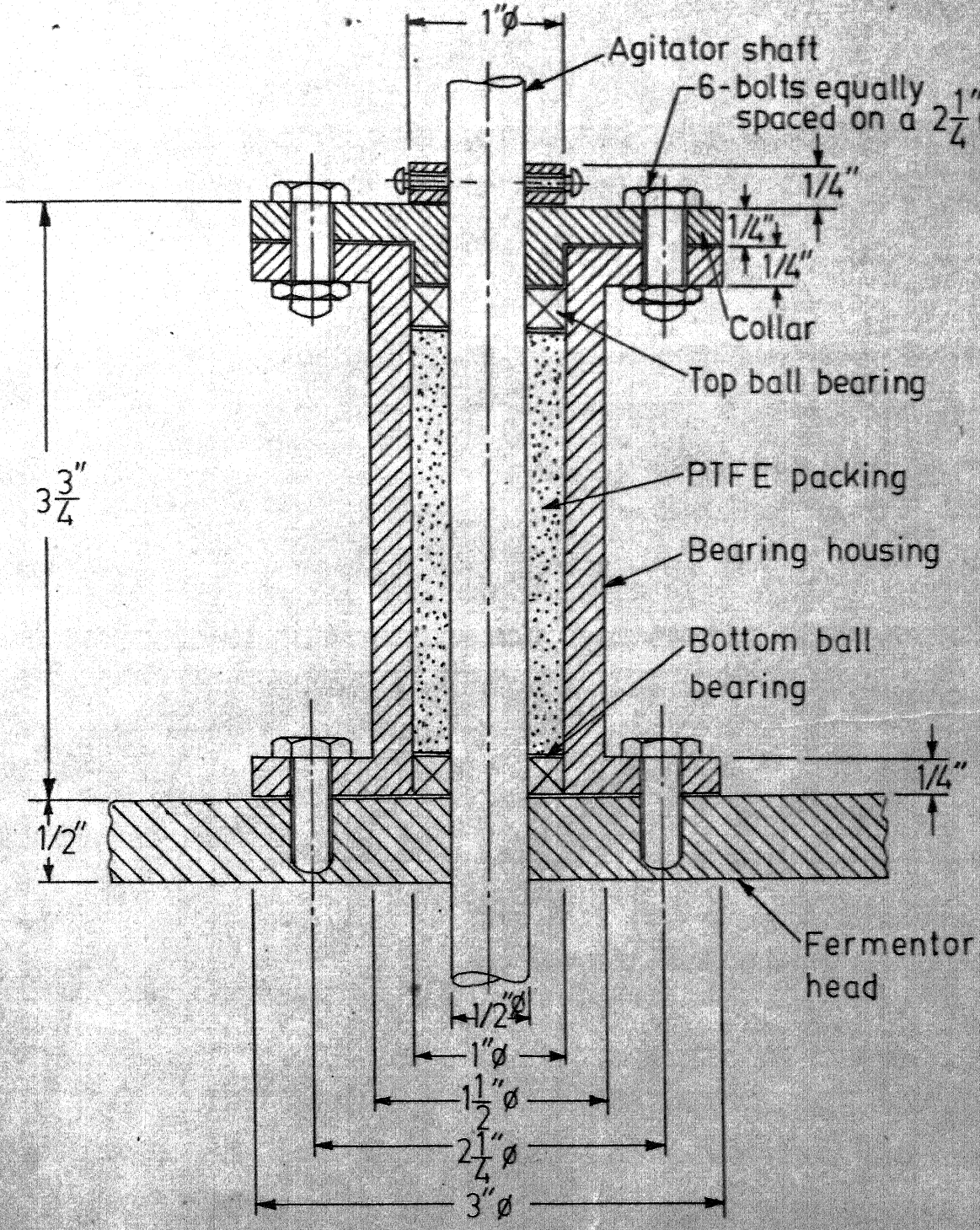

Fig. 6 - Sealed bearing assembly for agitator shaft.

the air through a single jet sparger placed close to the underside of the impeller. Usually multijet or porous spargers are not advantageous over single jet type, because the shear rates in the region of the impeller are generally sufficient to break up a jet of air into bubbles, which inturn provide an extensive area for oxygen transfer. Porous spargers are usually not used because of their tendency of getting blocked by micro-organisms.

(6) Cooling Coil:

For temperature control of the system, stainless steel cooling coils six in number each $5\frac{1}{2}$ inch diameter at a pitch of $1\frac{7}{16}$ inch with an effective heat transfer area equal to 5.21 inch^2 (33.6 cm^2) is placed inside the fermentor. The coil is connected to a constant temperature bath, which circulates cold water at an adjustable flow rate and at a constant temperature. In addition, the cooling coil also provides more intense mixing inside the vessel.

(7) Sterilization:

The fermentor is provided with a steam inlet port for sterilizing the inside of the fermentor along with all the inlet ports, pH-electrode, D-O_2 probe, thermocouple well etc.

The other alternative for sterilization could be to keep, whole of this fermentor assembly in an autoclave.

(8) Sterile Air System:

To reduce contaminations from the air supplied for aeration, the air supply has to be sterilized by causing a temperature rise by compressing the air and then passed through a sterilized cotton filter. To attain sufficient aeration, air is supplied at a pressure of 5.0 psig. Also it is advisable to put an additional filter on the air outlet to prevent infection in the event of back flow.

(9) Sampling:

Culture samples are removed through a sampling tube extending close to the bottom of the fermentor. With the vessel under positive pressure, samples are easily withdrawn by using a pipette.

Design details of various auxiliaries to the fermentor have been given in Appendix III.

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CHAPTER 5

HARDWARE DEVELOPMENT

Our system hardware is the interface between the fermentation unit and the IBM 1800. Complete hardware can be described as follows:

1. Measurement of the variables to be controlled
2. Signal noise
3. Signal transmission
4. Input interface
5. Output interface

5.1. Measurement of the Variables to be Controlled

Since in any fermentation unit, temperature and pH controls are very important. So, in this work, importance is given to their control.

5.1.1 Measurement of temperature:

Thermocouple is found to be the most suitable temperature sensing device in the temperature range of our interest and the accuracy requirement for measurement.

Following points are considered for the choice of suitable thermocouple:

1. Linearity in the range of operation.
2. Higher e.m.f./^{o}C change in the temperature, for higher accuracy in measurement.

3. Higher e.m.f. developed in the temperature range of operation. It reduces the complexities in signal transmission and amplifier design.

Considering all the above aspects, copper-constantan (Type T) thermocouple is chosen for temperature measurements.

While measuring temperature inside the fermentor, the hot junction of the thermocouple is introduced in a stainless steel thermowell filled with mercury, such that the hot end bead completely gets immersed in it to provide a better contact, while the cold junction is maintained at $0^{o}C$ by dipping it in an ice bath. The thermocouple output terminals are connected to the D.C. amplifier to amplify the low voltage (0 to 4 mV) signals to the level of 0 to 5V, so, that it gets easily transmitted through the cable, to the IBM 1800 system.

For millivolts versus temperature calibration refer to Appendix IV, (Figure 12.a).

5.1.2 pH measurement:

The pH-meter used, has also got the scale which reads the pH-value in millivolts. Since the e.m.f. is a linear functions of pH, so we can directly get the electrical signal from the pH-meter which is proportional to the pH of the solution in the fermentor.

In addition, pH-meter also consists of a D.C. amplifier, which amplifies the mV reading (in the range of -500 mV to

+500 mV) to 0 to 4 V d.c. output. Thus,we get an output d.c. voltage signal, proportional to the pH-of the solution which is directly transmitted through the cable running to the IBM 1800.

For voltage versus pH calibration, refer to Appendix V, (Figure 12.b).

5.2 Signal Noise:

In general the size of the process necessitates connection of transducer by long cables to the input unit of the computer. Quite often signal carrying cable is run close to the source of mains voltage, resulting in pickup of the leads. This pick up voltage is added to the transducer output. Usually for chemical processes the frequency of information signal is much small compared to the pick-up frequency. Thus by designing a suitable low pass active filter, between connecting cable and the multiplexer unit, pick-up can be attenuated sufficiently.

5.3 Signal Transmission [25]

From the process, the type of signals available are usually d.c. in nature. These signals can be transmitted over moderately large distances. One thousand feet is considered nominal maximum length. In any case the source must have the capability to drive the total loop impedance, which is the sum of the cable impedance and input impedance and also input impedance of the receiver.

However, high level d.c. signals are transmitted directly on signal cables. The shielded cable is normally used to avoid noise pick up.

Following factors were considered for the transmission of high level signal.

A.1. Ratio of receiver input impedance to the source of output impedance is kept greater than 1000. The D.C. amplifier output (output impedance 1000Ω) is connected to relay multiplexer (input impedance 10MΩ), so the above condition is satisfied.

A.2. The cable impedance is added to the source impedance to find the overall impedance. In present set-up, the input signal cable length is approximately 1000 feet in length, having an impedance much less than that of the source one.

A.3. The cable resistance and the capacitance combines together to form a RC-network, which acts like a filter. This filter should have a cut off frequency higher than the highest possible significant frequency of the signal output, so that the signals reach the computer without being seriously distorted and noise is reduced.

Transmission of Low Level Signals:

For the transmission of low level signals following points are helpful:

B.1. Transmission distance is kept as short as possible.

B.2. Signal cable shield is tied to the common point of the signal source and the shield starts right after the junction of the thermocouple and is maintained continuous upto the computer multiplexer relay terminals.

B.3. It should be ensured that neither the cable shield nor the signal ground is grounded at more than one point.

B.4. All the power lines etc. are kept away from the signal lines, so as to minimize the stray pick-up. If at all it becomes necessary to cross the signal lines and the power lines, they should cut at a right angle.

5.4. Input Interface:

In case of temperature measurements the magnitude of the thermocouple output voltages is very small (of the order of 0-4 mV in the temperature range of 0-100^{o}C). Since the input to the computer must be of the order of volts (1-5 volts), high gain differential amplifier is used at input interface. The input impedance of the amplifier must be high enough to avoid loading of the thermocouple signals.

However, in the case of pH measurements d.c. signals are of order of 1-2 volts i.e. in desired range of operation, so, no amplifier is needed and the output from pH-meter is directly connected to the active low pass noise filter and output of noise filter is connected to relay multiplexer terminals.

5.4.1 D.C. Amplifier:

The following are the requirements of the D.C. amplifier to amplify thermocouple signals and other low level signals.

(a) High input and low output impedance.

(b) High common mode rejection

(c) High stability

(d) Good linearity in the range of operation.

For this purpose a variable gain d.c. amplifier is used so that the same can be used for other transducers output to be coupled to computer input. The specifications decided for amplifier are:

1. Floating input and floating output. (Since the relay multiplexer requires the floating input).
2. Zero offset arrangement
3. Gain settings of ± 2000, ± 1000, ± 500, ±200, ±100, ±50, ±20, and ± 10 are available, so that all types of transducer output can be matched to computer input requirement.
4. Powered by A.C. mains supply.

This amplifier can be used for only one signal and each low level signal requires a separate unit.

5.4.2 Active low-pass noise filter:

As discussed earlier, this unit is required to reject any high frequency signals, picked up by the transmission line

from time to time. This noise filter is connected in between the output from the d.c. amplifier and the input terminals of the relay multiplexer.

We use a noise filter having following specifications:

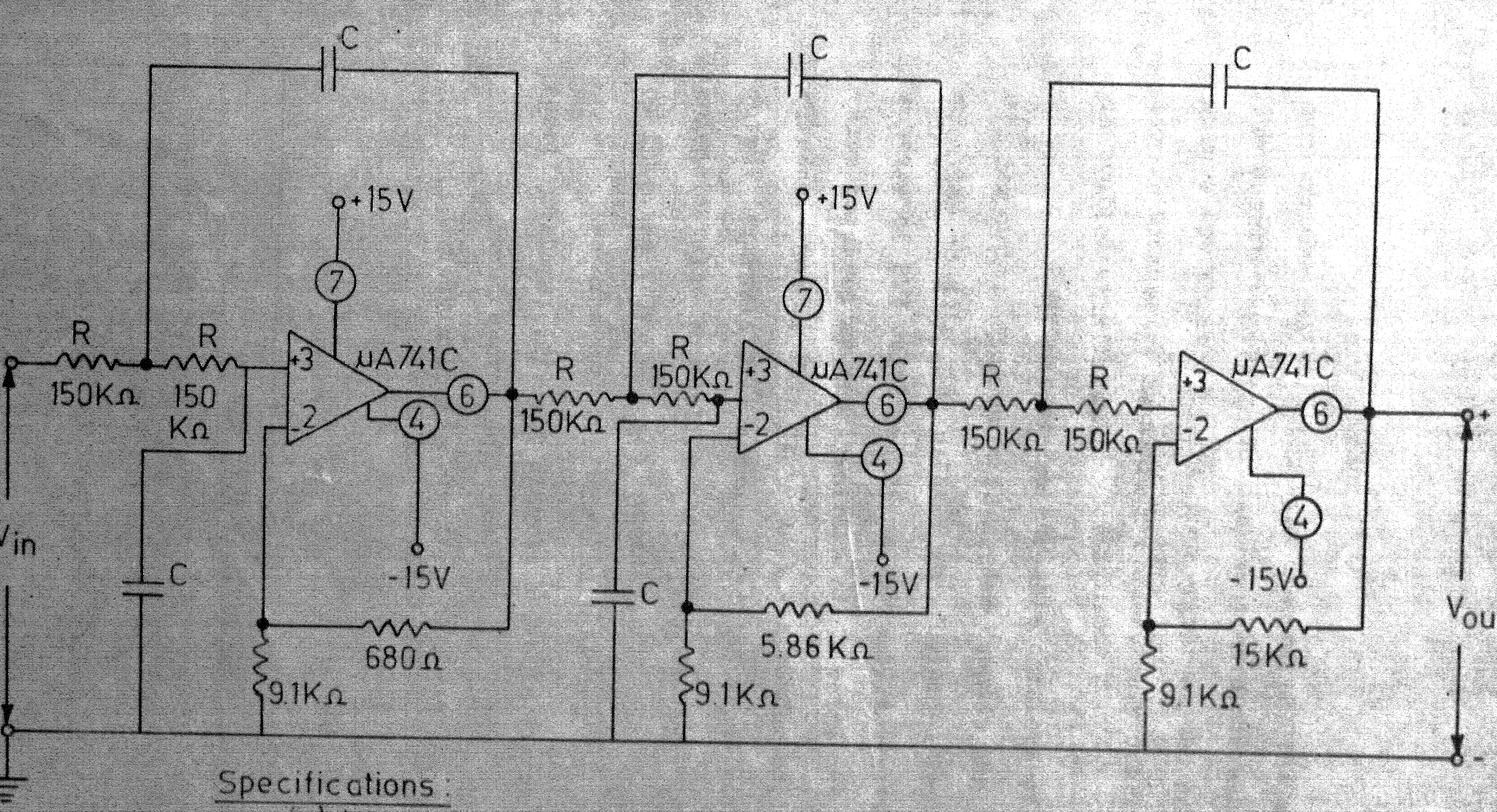
Cut off frequency	= 2 Hz
Overall gain	= 4.2
An output voltage pick up	= 40 mV
Input voltage	= ± 15V

Figure 7 shows a circuit diagram for this active low pass noise filter.

5.5 Output Interface:

Voltage actuated solenoid valve is used for controlling the flow rate of cold water in the case of temperature control, while pH was controlled by the addition of base/acid through another solenoid valve.

Since the valves are of on-off type, normally closed one, so once the output signal reaches them (after execution of program and comparing the measured value with set value) from the computer, they get opened and remains open until the set value reaches inside the fermentor (within the error limit of temperature and pH-value). Once the required temperature and pH are attained inside the fermentor, no signal comes from computer and valves get automatically closed. So, in this way at an specified time interval (30 seconds in case of



Specifications :

(a) Low pass filter = 36 dB / octave frequency roll off.

(b) Voltage gain = 4.2

(c) fc = 2 Hz C = 0.47μf , 10% tolerance, 400 V D.C.

R = 150 KΩ , 1% tolerance, 1/2 W

Fig. 7 - Active low pass noise filter circuit.

temperature and 10 seconds in case of pH) the signal goes to the computer passing through input hardware and simultaneously values are printed by the type writer at the computer console time. Because of hardware problems, only one variable is controlled at a time.

Output system hardware consists of a valve driving unit. This unit is needed because the output signal from the computer is in the form of digital level, having fixed voltage of 2.7 volts and 26 mA current, while the solenoid valve requires an input voltage of 12 volts and 1.3 amps. of current. Hence, this drive unit was connected in between the output terminals from computer and the input points of the solenoid valve to amplify the output signal to such a valve, so that the valve may get actuated.

We used a valve drive unit having the following specifications; (Circuit diagram shown in Figure 8).

Input operating voltage	= ± 12V
Output signal	= 1.3 Amp.
Input signal	= 2.7 V
Current	= 26 mA

Specifications of Solenoid Valve:

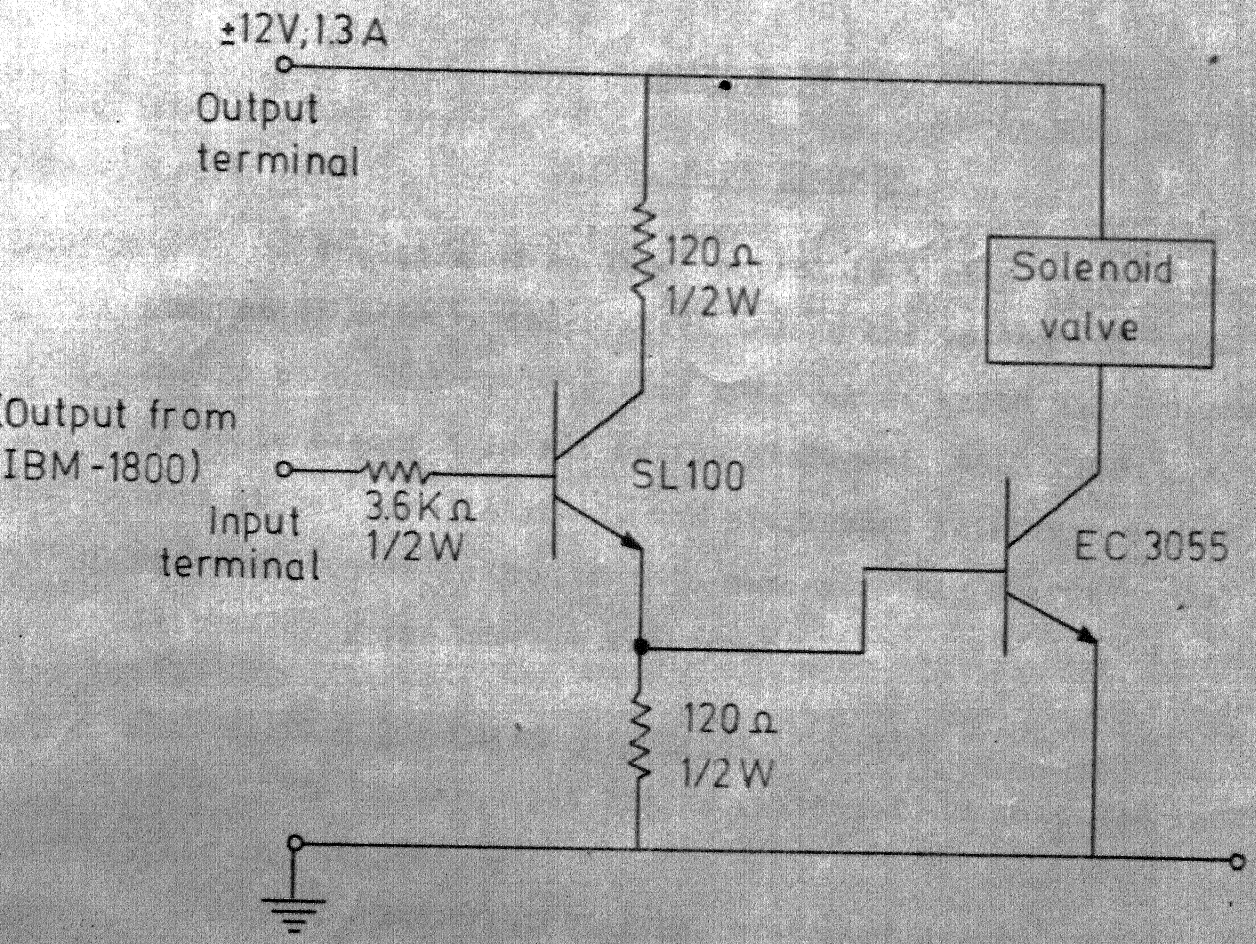
Two way, 1/4" BSP, 1/4" orifice,

Normally closed

Pressure 0-150 psi

Direct acting, mini solenoid valves with stainless steel body

Voltage 12 V D.C.



Specifications:

Input - +2.5V,300μA

Output - +12V, 1.3A

Operating voltage - 12 V D.C.

Fig. 8 - Pneumatic valve drive circuit.

CHAPTER 6

SOFTWARE DEVELOPMENT

The idea of on-line control is that periodically, the program to measure temperature and pH and to control them should be executed. The time interval at which the program is to be executed is set by the statement 'CALL TIMER', so that at set time interval, the programmed interrupt occurs and the program is loaded into memory. This operation is initiated by the process interrupt.

Initiation and Program Execution:

The program [26,27] is initiated and executed in following stages:

1. COMMON/INSKEL/ITEMP statement describes that ITEMP will receive one analog signal after conversion by ADC.

2. DATA K/Z8000/statement is used for preventing any other interrupt to come at zero level.

3. CALL UNMK(-1,-1) unmasks all levels of interrupts which might have masked because of other process operations.

4. CALL SETCL(II) is used for the initial time setting of the real time clock, where I is expressed in thousandths of an hour.

5. CALL MASK(K,-1) masks the zero level.

6. CALL TIMER (NAME,I,NUMB) controls the first two timers in the memory locations 0004 and 0005. The argument

NAME is the nameof a user subroutine to be executed at the end of the specified time period. I is an integer expression or variable equal to '1' if the first timer is used and '2' if second is used. NUMB is the time interval between two successive interrupts in thousands of seconds. In thisprogram, subroutine SCAN is executed in EXTERNAL statement, timer #2 is used and interrupts are given at a interval of 30 seconds.

7. IF(LD(5))41,42,42 is a test statement for timer. It will transfer control to statement # 41 if the timer # 5 in memory statement (0005) is busy or else to statement #42, if the timer is not busy.

8. Now once the interrupt is received, the program is queued depending on its priority of a process job is currently under execution. At the completion of this job, the core load waiting at the top of the queue at this time is loaded into the memory.

9. If a non process program is in execution, the job in execution is saved on to the disk in the interrupt save area and the main line program, which is stored in the disk in core image form is loaded in the core memory.

10. Now once the analog value of temperature gets converted to the temperature, it asks for the set value, and finds the difference of the set value and measured one and if difference is more than or equal to '1' the digital output level is generated, which gives the signal for valve closure or

opening. Statement IDATA(1)=0 transfers control for valve closure, while I DATA(1)=1 for opening.

11. CALL CLOCK(I) reads the current clock value in hours and thousandths of an hour into the integer variable 'I' for interrogation by user's program.

12. CALL LEVEL (I) causes the computer to transfer control to a subroutine assigned to interrupt level 'I' which is '11' in our case.

13. CALL AIP (11000,ITEMP,10) statement specifies that 14 bit resolution is to be used to read an analog signal from first ADC without external synchronization. The signal is to be read from a relay multiplexer wired to analog point 10, and the converted reading is to be stored in the variable name ITEMP.

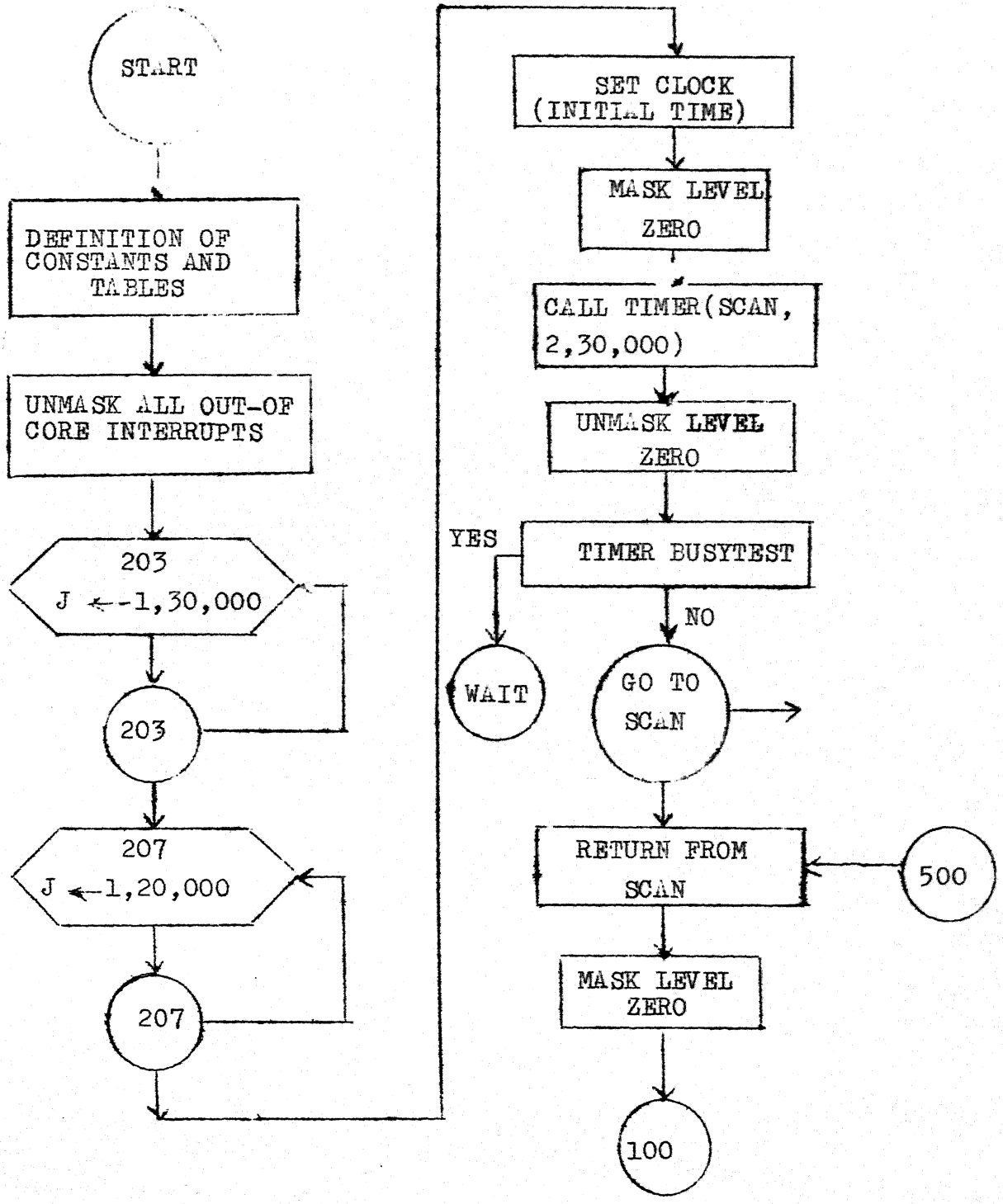
14. CALL AIP(0,ITEST) statement is used to test whether analog input point is busy or not.

15. CALL INTEX - is usually the last stement to be executed in an interrupt program, and it stands for call interrupt exit.

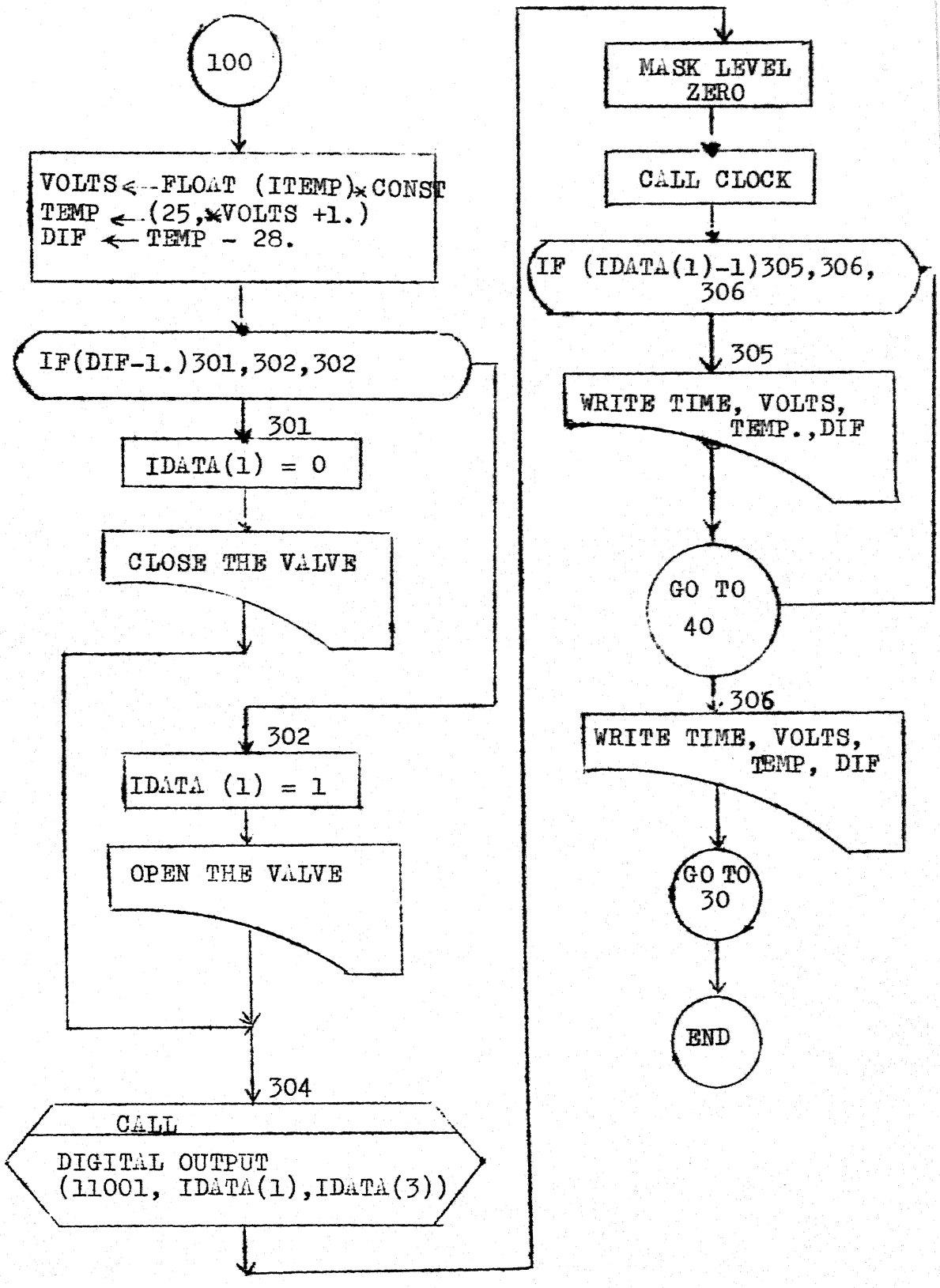
Now, exactly on the same lines the control program for 'pH' has been written and is stored in memory. Program listings for 'temperature' and 'pH' control programs are given in Appendix VII. Also figure 9 represents a flow diagram for temperature control.

+ + + + +

FIGURE 9: FLOW DIAGRAM FOR TEMPERATURE CONTROL PROGRAM



Contd.


100
VOLTS ← FLOAT (ITEMP) * CONST
TEMP ← (25, * VOLTS +1.)
DIF ← TEMP - 28.
IF(DIF-1.)301,302,302
301
IDATA(1) = 0
CLOSE THE VALVE
302
IDATA (1) = 1
OPEN THE VALVE
304
CALL
DIGITAL OUTPUT
(11001, IDATA(1),IDATA(3))
MASK LEVEL ZERO
CALL CLOCK
IF (IDATA(1)-1)305,306, 306
305
WRITE TIME, VOLTS, TEMP.,DIF
GO TO 40
306
WRITE TIME, VOLTS, TEMP, DIF
GO TO 30
END

SUBROUTINE SCAN

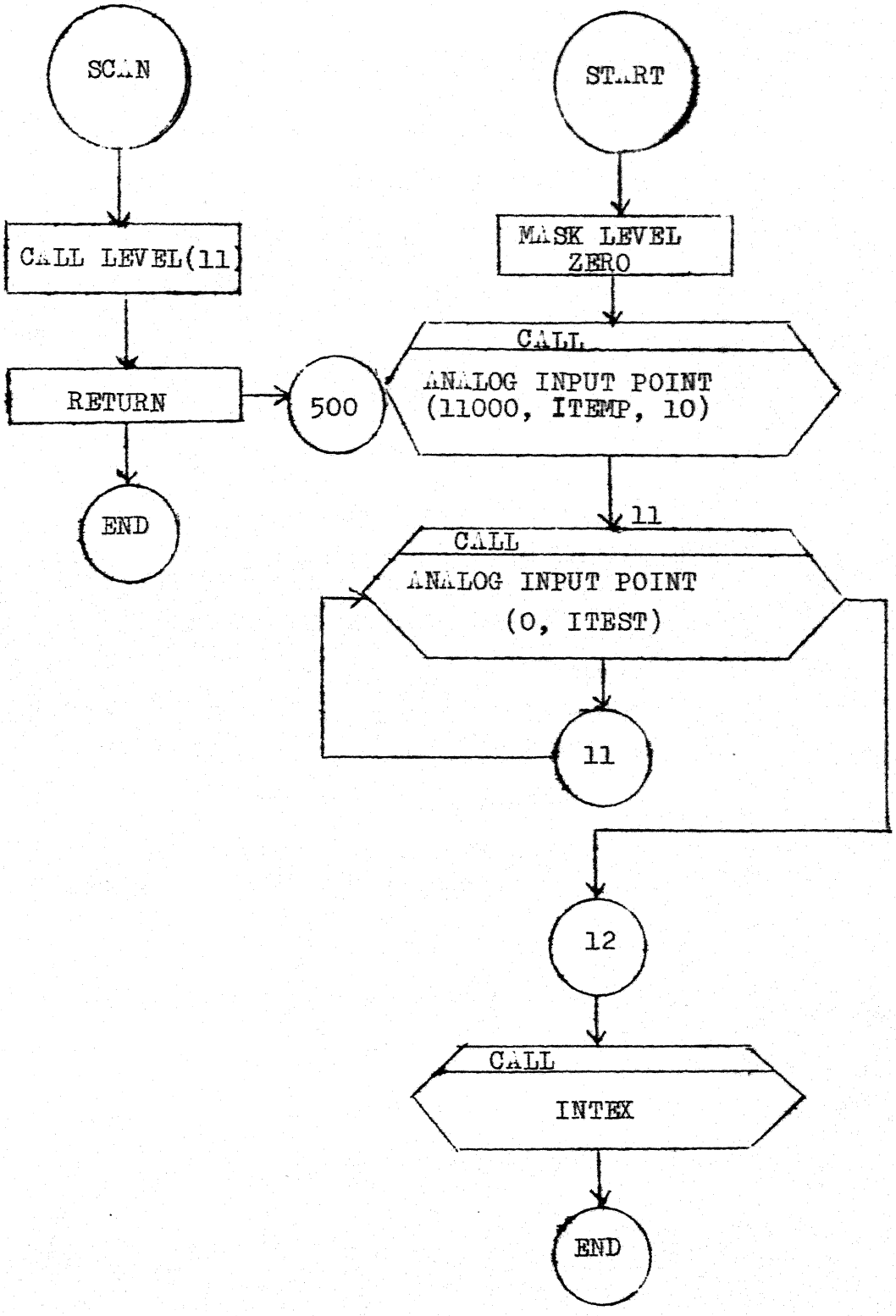


FIGURE 9

CHAPTER 7

RESULTS AND DISCUSSION

Experimental results regarding yeast growth and decrease in sugar concentration with time are tabulated in Appendix VIII The results of temperature and pH-control of the fermentor are given in Appendix IX.

Table 5 gives the percent sugar concentration in the broth at different intervals of time. Yields of Baker's yeast obtained at different intervals of time during fermentation are also tabulated in Table 6.

Figure 10 shows the gradual decrease in the sugar concentration of the fermentor liquid with time. The amount of yeast present in the fermentor at any time is shown in Figure

The reaction and the control part of the fermentation are discussed as follows:

7.1 Fermentation Reaction

It was found that as the reaction proceeds, there is a decrease in the sugar concentration, and an increase in the total yeast content. This confirms that the fermentation was well going in the fermentor.

It was also noticed that during first one and a half hour the reaction rate increases and attains a maximum yield of 38 per cent (based on substrate concentration) as shown in Table 6. However, after this time period the product yield

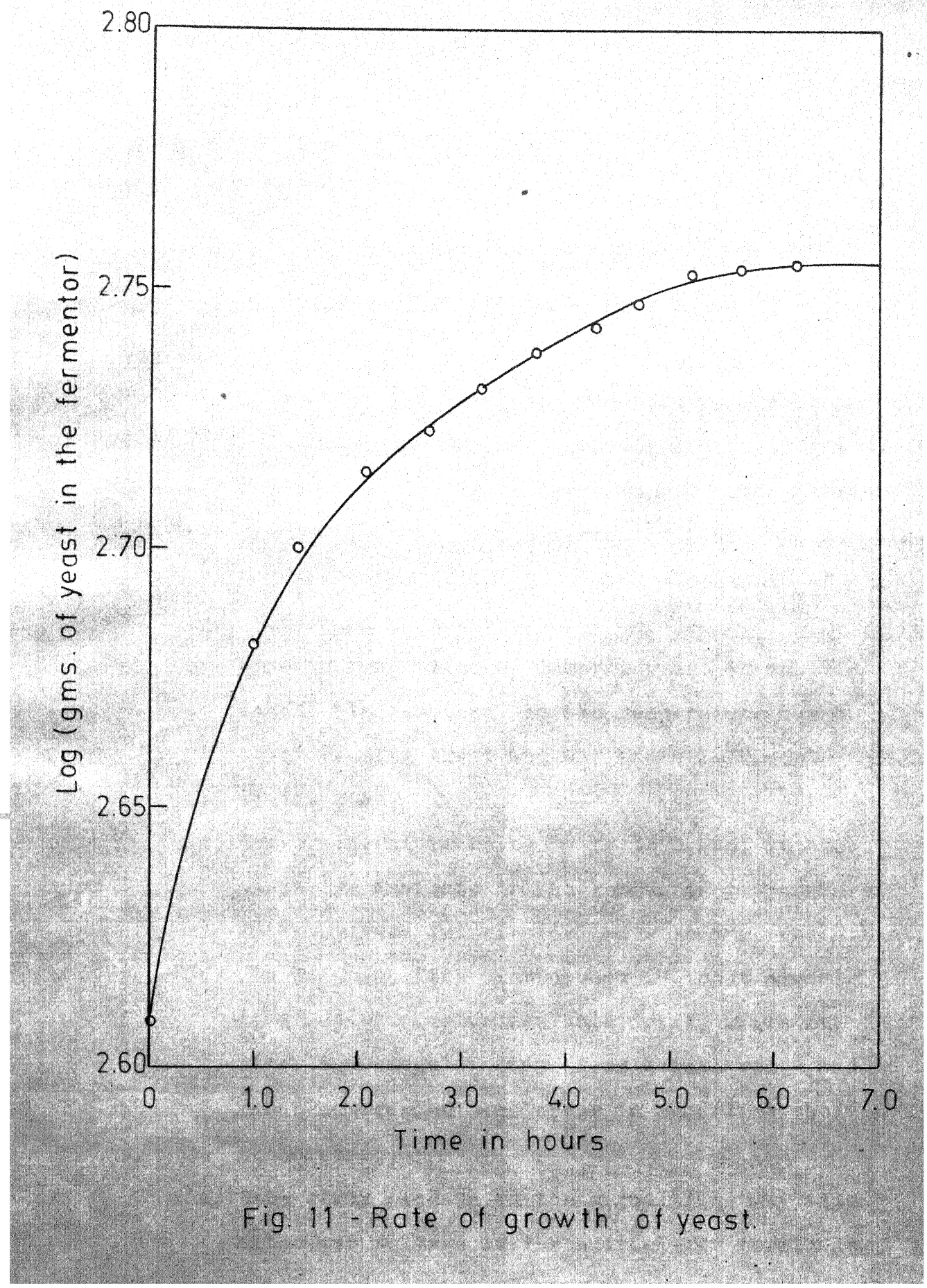


Fig. 11 - Rate of growth of yeast.

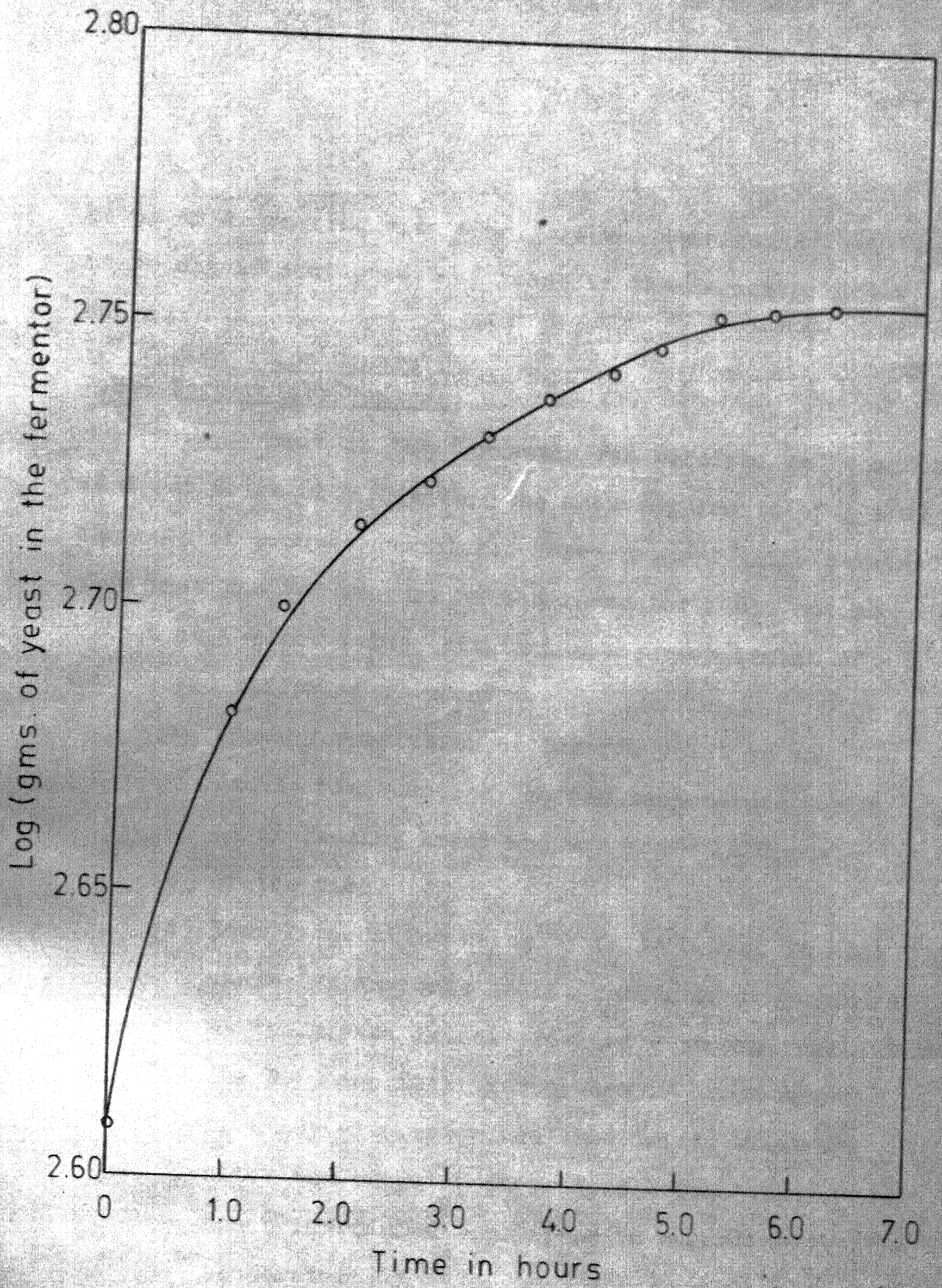


Fig. 11 - Rate of growth of yeast.

keeps on decreasing with time. It was also found that there is no significant growth of yeast in the fermentor after six hours.

7.2 Fermentation Control:

7.2.1 Temperature control:

Temperature in the fermentor was required to be controll at a set valve of 28°C(within the accuracy limits of ± 1°C) wi the help of process computer. However, the results obtained, show that the temperature of the fermentor could not be maintained at the set value (within the accuracy limits of ± 1°C) due to the following reasons:

[a] Since fermentation of Baker's yeast is an exothermic reaction, so the temperature has an increasing trend and was above 28°C, most of the time.

[b] Room temperature being 35 to 36°C, so, it also causes an increase in the fermentor temperature as the system is not provided with any insulations.

[c] The D.C. amplifier introduced in input signal line may also introduce some drift, which may alter the output voltage from actual value and hence introduces some error in recording the temperature.

[d] Some stray pick-up in the signal line may also alter the voltage at the multiplexer terminals.

A suitable designed R-C filter will attenuate the pick up to considerable standard.

The obtained data logging shows that the temperature in the fermentor could be maintained around 28°C within the accuracy limits of ± 1.5°C. Data logging obtained during temperature control is given in the Table 7 (Appendix IX).

7.2.2 pH Control:

pH of the fermentor broth was required to be controlled at a set value of 4.8 (within the accuracy limits of ± 0.1 pH unit). The results obtained show that pH-control went very we within the set limits of control. Whatever variations are there, they are due to following reasons:

[a] There is always some time lag in the system due to time lag in the pH-meter and probe to give stable reading.

[b] Some stray pick up in the signal line may also alter the voltage at the multiplexer terminals.

The obtained data logging results show that the pH in the fermentor was very well maintained within the accuracy limits of ± 0.1 pH unit. The data logging obtained during pH-control is given in the Table 8 (Appendix IX).

+ + + + +

CHAPTER 8

CONCLUSION AND RECOMMENDATIONS

Conclusion:

1. A 14 litres capacity laboratory fermentor was designed and fabricated and was operated successfully except some minor problems.
2. A maximum yield of 38 per cent of yeast was attained in the Baker's yeast fermentation. Lower yields were resulted due to improper sterilization of the auxiliaries, pipe lines carrying reagents, interior parts of the fermentor and contamination during aeration and inoculation.
3. The interface between the fermentor and the process computer was well designed and thus temperature and pH-control were achieved under prescribed accuracy limits of control.

Recommendations:

1. There should be proper provision for independent sterilization of pipe lines, and various auxiliaries inside the fermentor with steam.
2. The fermentor must be provided with a steam sterilizable sampling port for quick and ascptic withdrawal of the samples.

3. A constant agitator speed must be maintained by using a speed controller as the viscosity of the culture medium changes during the fermentation.
4. Provisions for the control of dissolved oxygen concentration, antifoam addition and agitator speed should also be provided for the complete control of the fermentor.
5. By providing all the controls as discussed above, an optimal control model for each variable can be developed separately.
6. An optimal control model could be developed by considering effects and interactions of all the process variables, which further require more detailed knowledge of metabolic activities of cells during fermentation and nature of interactions between different variables.

+ + + + +

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APPENDIX I

METHOD FOR DETERMINING THE SUBMICRO-AMOUNTS OF SUGAR [28](Phenol-Sulfuric Acid Method)

2 ml of sugar solution (containing between 10-70 micro grams of sugar) is pipetted into a tube, and 1 ml of 5 per cent phenol in water is added and then 5 ml of concentrated H_2SO_4 is added rapidly and kept for 10 minutes and then tube is shaken and placed in a bath (at 25°C) for 10 to 20 minutes. Now the absorbance of this solution is measured. In this case the color is now stable for several hours and reading may be taken after some time, if necessary. While measuring the absorbance of the solution, wave length is set at 480 mμ for sugar measurements.

Blanks are prepared by substituting distilled water for sugar solution. The amount of sugar may then be determined by reference to a standard calibration curve previously constructed by making several standard sugar solutions of known concentrations and then measuring their absorbance and finally a calibration curve is plotted between micrograms of sugar/ml vs absorbance, as shown in Figure 12(c).

This method can be used to give reliable estimations of the sugar content of pure solutions. The colours produced are unusually stable, and possesses a definite absorption peak. The amount of colour produced at a constant phenol concentration is proportional to the amount of sugar present in the solution.

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APPENDIX II

METHOD FOR DETERMINING YEAST/CELL GROWTH

10 ml of the sample solution is pipetted out after well shaking the flask and is taken in a test tube. Then, it is washed with distilled water for 3-4 times and then centrifuged at 1000 rpm for 5 minutes. Then, the upper liquid layer is decanted and the tube is kept in an oven maintained at constant temperature of 60°C and each sample is dried for approximately 20 hours.

Then dried yeast is removed from the tube and weighed. Thus, we get the amount of yeast present per 10 ml of the sample. In this way the total yeast present in the fermentor at particular time can be estimated and a graph can be obtained by plotting time in hrs. vs log (grams yeast in fermentor).

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APPENDIX III

TABLE - 1

DESIGN DETAILS, MATERIALS OF CONSTRUCTION OF VARIOUS AUXILIARIES TO THE FERMENTOR

S.No.	Item	Length (inches)	Diameter (inches)		Wall/Plate thickness (inches)	Material of construction	Quantity	Remarks
			O.D.	I.D.				
1.	Fermentor vessel	18	$8\frac{11}{16}$	$8\frac{1}{16}$	5/8	Corning glass	1	Cylindrical vessel clos at one end.
2.	Top plate	-	$12\frac{1}{2}$	-	1/2	Stainless steel	1	Top plate has an O-ring fitted in the lower sid to make the system leak proof.
3.	Base plate	-	$12\frac{1}{2}$	-	1/4	Stainless steel	1	-
4.	Tie rods	22	1/2	-	-	Stainless steel	6	The rods are used for tightening top and bott plates.
5.	Thermocouple well	$11\frac{3}{8}$	1/2	3/8	1/16	Stainless steel	1	Closed at one end and filled with mercury for better contact.
6.	Cooling coil	14	1/4	1/8	1/8	Stainless steel	1	Having six coils, each $5\frac{1}{2}$ inch at a pitch of 23/16" using water as coolant, and having hea transfer area = 5.21 in

S.No.	Item	Length (inches)	Diameter (inches)		Wall/Plate thickness (inches)	Material of construction	Quantity	Remarks
			O.D.	I.D.				
7.	Agitator shaft	24	1/2	-	-	Stainless steel	1	-
8.	Bearing House	-	-	-	-	Stainless steel	1	For design details, refer Figure 6.
9.	Impeller	-	3	-	1/8	Stainless steel	2	Flat blade turbine type.
10.	Baffles and reinforcing ring	17½	Reinforcing ring $7\frac{7}{16}$	- $6\frac{15}{16}$	1/8 -	Stainless steel	4	Reinforcing ring is u to keep the baffles i position.
11.	Sampling port	16	1/2	3/8	1/16	Stainless steel	1	-
12.	Sterile air Inlet	24	1/2	3/8	1/16	Stainless steel	1	Having a single jet sparger at the outlet
13,	Auxiliary liquid addition port	10	1/2	3/8	1/16	Stainless steel	1	-
14.	Acid/base addition port	10	1/2	3/8	1/16	Stainless steel	1	-
15.	Antifoam addition port	8	1/2	3/8	1/16	Stainless steel	1	-
16.	Substrate addition port	12	1/2	3/8	1/16	Stainless steel	1	-
17.	Inoculation port	10	1/2	3/8	1/16	Stainless steel	1	-

Table 1 (contd)

S.No.	Item	Length (inches)	Diameter (inches) O.D.	Diameter (inches) I.D.	Wall/Plate thickness (inches)	Material of construction	Quantity	Remarks
18.	Steam inlet	24	1/2	3/8	1/16	Stainless steel	1	
19.	pH-probe seal and probe	-	-	-	-	Stainless steel	1	For Design details refer to Fig.4
20.	D-O_2 probe seal and probe	-	-	-	-	Stainless steel	1	For design details refer to Fig.5

APPENDIX IV

CALIBRATION OF THERMOCOUPLE

Thermocouple calibration is required to determine the millivolt value at a particular temperature i.e. it is a plot between millivolts vs temperature (^{o}C). By this calibration curve, we find the best possible mathematical relationship and this relationship is required in the system software development.

Following table gives the e.m.f. values at a particular temperature. Figure 12(a) shows the calibration curve for thermocouple.

TABLE 2

MILLIVOLTS vs TEMPERATURE

Thermocouple - Copper constantan (Type T)
Reference junction temperature = ^{o}C
Room temperature = $22^{o}C$

S.No.	Temperature, (^{o}C)	e.m.f. (millivolts)
1	98.4	4.29
2	88.0	3.748
3	80.0	3.39
4	75.0	3.15
5	69.5	2.892
6	59.5	2.407
7	48.2	1.882

S.No.	Temperature, (^{o}C)	e.m.f.(millivolts)
8	42.5	1.641
9	39.0	1.515
10	35.2	1.354
11	31.0	1.186
12	30.0	1.145
13	28.5	1.112
14	27.0	1.066
15	26.0	1.000
16	21.3	0.833
17	18.0	0.686
18	15.0	0.586
19	10.0	0.390

Equation of the straight line TEMP = 25.* MILLIVOLTS +1.

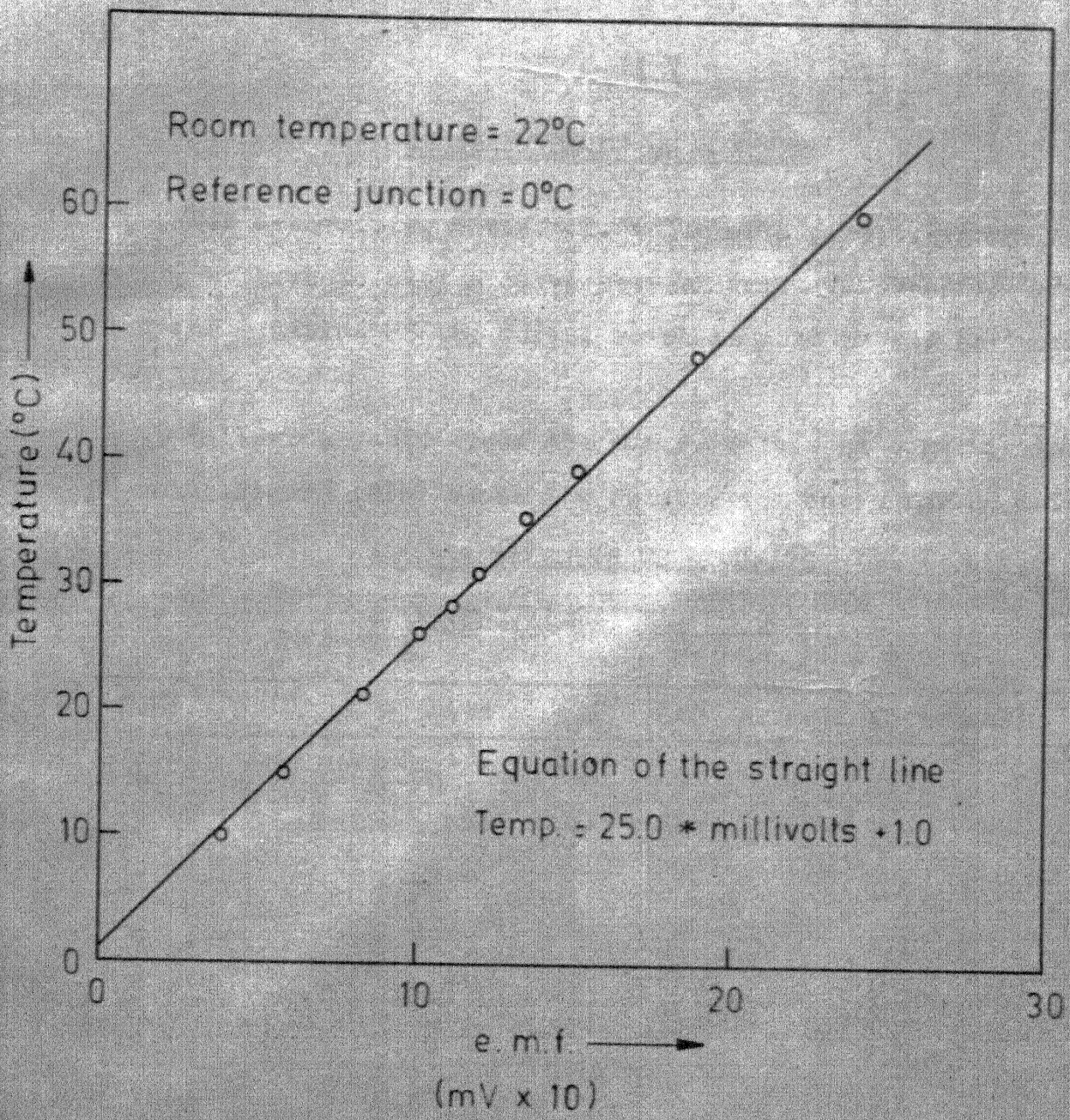

Fig.12(a) - Calibration plot for copper-constantan thermocouple

APPENDIX V

CALIBRATION OF pH - METER

For determining the e.m.f. value at a particular pH-value, we have to plot a curve between volts vs pH-units and find the equation of the curve, which is used in the software development.

Following table gives the e.m.f. values at a particular pH-value.Figure 12(b) shows the calibration curve for pH-meter.

TABLE 3: VOLTS VS pH-UNITS

ROOM TEMPERATURE= 33°C

S.No.	pH - units	e.m.f. (volts)
1	2.00	1.50
2	3.94	1.78
3	4.86	1.89
4	6.55	2.12
5	7.75	2.30
6	9.40	2.50
7	9.68	2.55
8	10.78	2.70
9	12.00	2.86

Equation of the straight line: pH = 7.312 *VOLTS - 8.983

+++++

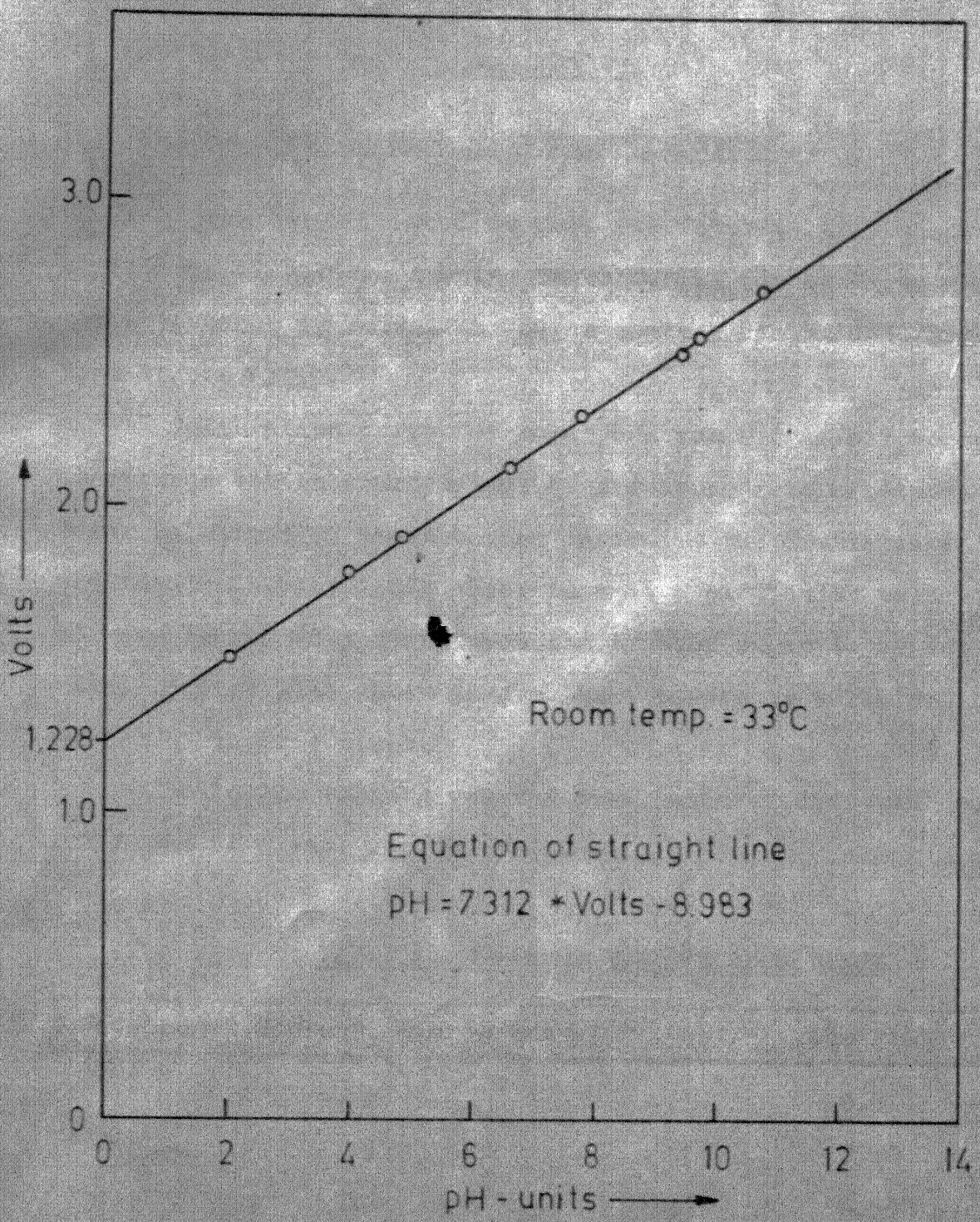

Fig 12(b)- Calibration plot for pH-meter.

APPENDIX VI

CALIBRATION OF SPECTROPHOTOMETER

Spectronic-20 model is used for determining the absorbance of various samples taken during fermentation. Once the absorbance is known, the sugar content can be determined from Figure 12(c).

Calibration curve for sugar content ('μ'gms/ml) versus absorbance can be constructed, by preparing several standard sugar solutions of known concentration in the distilled water and then measuring their absorbance at a particular value of wave length (depending upon the type of sugar to be estimated). Distilled water is used as a reference solution having no sugar content.

Following table gives the absorbance of different sugar solutions at a wave length of 480 mμ.

TABLE 4: SUGAR CONTENT VS ABSORBANCE

S.No.	Sugar Content, Micrograms ml^{-1}	Absorbance
1	0	0.0
2	5	0.38
3	10	0.508
4	20	0.528
5	30	0.66

Cont.

S.No.	Sugar Content, Micrograms, ml^{-1}	Absorbance
6	40	0.74
7	50	0.76
8	60	0.85
9	70	0.86
10	80	0.95
11	90	1.07
12	100	1.12
13	120	1.20
14	140	1.25
15	180	1.43
16	200	1.50

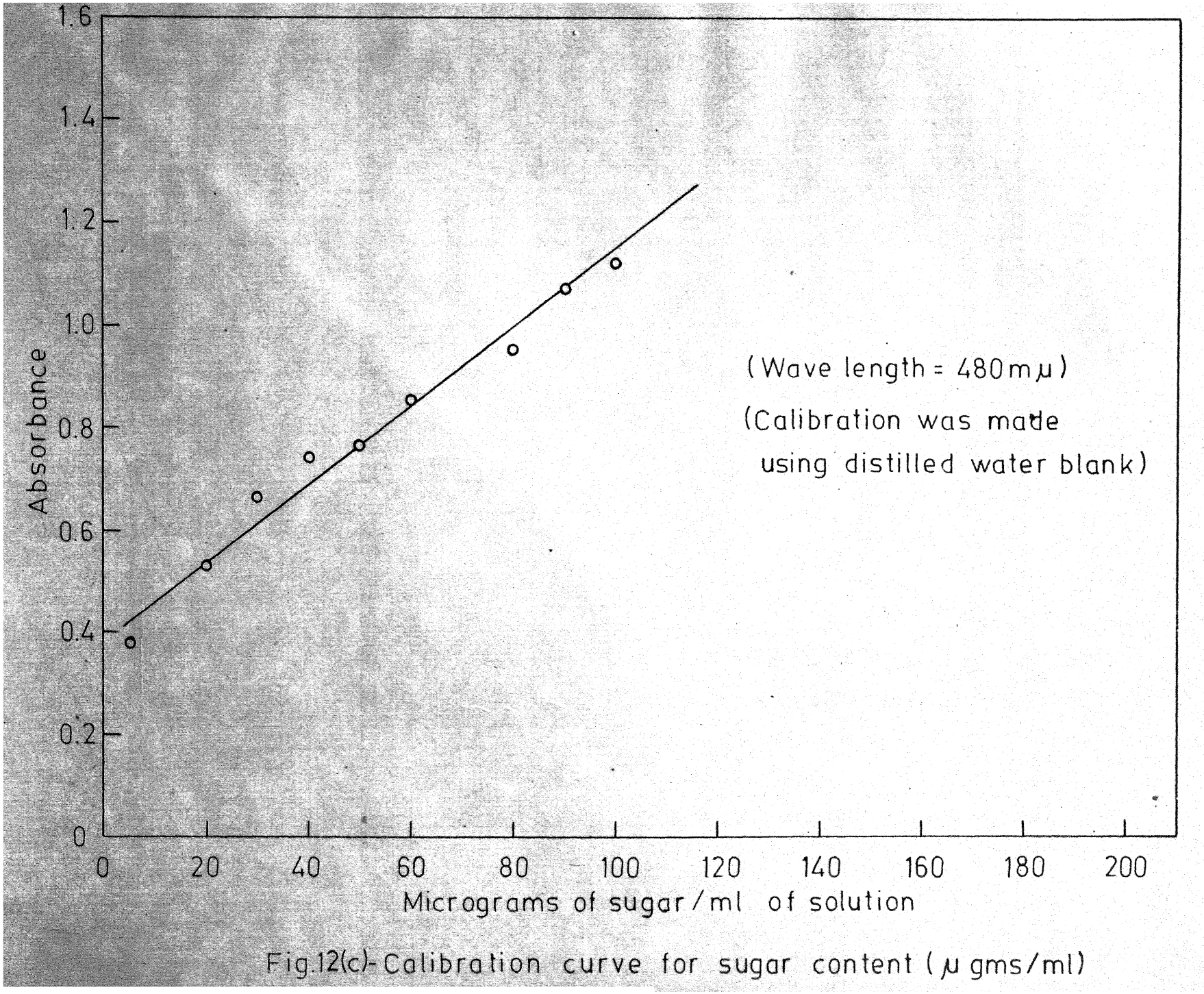

Fig.12(c)- Calibration curve for sugar content (μ gms/ml)

APPENDIX VII

(A) COMPUTER PROGRAM FOR TEMPRATURE CONTROL

```
// JOB
// DUP
*DELETE   M          GL2
// JOB
// FOR GOEL
*IOCS(KEYBOARD,TYPEWRITER)
*ONE WORD INTEGERS
      EXTERNAL SCAN
      DIMENSION IDATA(3)
      COMMON/INSKEL/ITEMP
       DATAK/Z8000/
      IDATA(3)=2
      IDATA(2)=124
       CONST=5./32767.
       CALL UNMK(-1,-1)
       WRITE(1,201)
       DO 203J=1,30000
203    CONTINUE
       WRITE(1,202)
       DO207KKK=1,6
       DO207 J=1,20000
207    CONTINUE
       READ(2,204)II
       CALL SETCL(II)
       WRITE(1,205)
30     CALL MASK(K,-1)
      CALL TIMER(SCAN,2,30000)
       CALL UNMK(K,-1)
41    IF(LD(5))   41,42,42
42     CALL MASK(K,-1)
      VOLTS=FLOAT(ITEMP)*CONST
      VOLTS=VOLTS*4.0
      TEMP=25.*VOLTS+1.
      DIF=TEMP-28.0
      IF(DIF-1.0)301,302,302
301   IDATA(1)=0
      GOTO 304
302   IDATA(1)=1
304    CALL DO (11001,IDATA(1),IDATA(3))
       CALL MASK(K,-1)
       CALL CLOCK(ITIME)
       IF(IDATA(1)-1)305,306,306
305    WRITE   (1,101)ITIME,VOLTS,TEMP,DIF
       GOTO40
306    WRITE(1,102)ITIME,VOLTS,TEMP,DIF
40      CONTINUE
      GO TO 30
201    FORMAT('**** GOEL''S PROGRAM FOR CONTROL OF FERMENTOR''S TEMP****
     1'/)
202    FORMAT('***WRITE TIME IN HOURS AND  MILLI HOURS IN I5 FORMAT**'/)
204     FORMAT(I5)
205    FORMAT(/// 6X,'TIME'13X,'VOLTS'11X,'TEMP',6X,'DIF',6X,'COMMENT'/)
102    FORMAT(/5X,I5,8X,F10.3,5X,F10.3,2X,F8.3,'    OPEN  THE VALVE')
101    FORMAT(/5X,I5,8X,F10.3,5X,F10.3,2X,F8.3,'    CLOSE THE VALVE')
```

```
      END

// FOR SCAN
      SUBROUTINE SCAN
       CALL LEVEL(11)
        RETURN
         END
*STORECI   M          GL2    GOEL   GL2
*CCEND


// JOB
// DUP
*DELETE    I          WS     WS1          2411

// FOR WS
*ONE WORD INTEGERS
      COMMON/INSKEL/ITEMP
       DATAK/Z8000/
       CALL MASK(K,-1)
      CALL AIP(11000,ITEMP,10)
11    CALL AIP(0,ITEST)
       GOTO(11,12),ITEST
12    CONTINUE
       CALL INTEX
      END
*STORECI   I          WS1    WS
*CCEND
```

```
// JOB
// DUP
*DELETE    M          PL2
// JOB
// FOR GOEL
*IOCS(KEYBOARD,TYPEWRITER)
*ONE WORD INTEGERS
      EKTERNAL SCAN
      DIMENSION IDATA(3)
      COMMON/INSKEL/IPH
       DATAK/Z8000/
      IDATA(3)=2
      IDATA(2)=124
       CONST=5./32767.
       CALL UNMK(-1,-1)
       WRITE(1,201)
       DO 203J=1,30000
203    CONTINUE
       WRITE(1,202)
      DO207 KKK=1,6
       DO207 J=1,20000
207    CONTINUE
       READ(2,204)II
       CALL SETCL(II)
       WRITE(1,205)
30     CALL MASK(K,-1)
      CALL TIMER(SCAN,2,10000)
       CALL UNMK(K,-1)
41    IF(LD(5))   41,42,42
42     CALL MASK(K,-1)
      VOLTS=FLOAT(IPH)*CONST
      PH=7.312*VOLTS-8.983
       DIF=4.8-PH
      IF(DIF-0.10)301,302,302
301   IDATA(1)=0
      GOTO 304
302   IDATA(1)=1
304    CALL DO (11001,IDATA(1),IDATA(3))
       CALL MASK(K,-1)
       CALL CLOCK(ITIME)
       IF(IDATA(1)-1)305,306,306
305    WRITE   (1,101)ITIME,VOLTS,PH,DIF
       GOTO40
306    WRITE(1,102)ITIME,VOLTS,PH,DIF
40      CONTINUE
      GO TO 30
201    FORMAT('**** GOEL''S PROGRAM FOR CONTROL OF FERMENTOR BROTH PH **
     1*/)
202    FORMAT('***WRITE TIME IN HOURS AND  MILLI HOURS IN I5 FORMAT**'/)
204     FORMAT(I5)
205    FORMAT(/// 6X,'TIME'13X,'VOLTS'11X,'PH',6X,'DIF',6X,'COMMENT'/)
101    FORMAT(/5X,I5,8K,F10.3,5X,F10.3,F8.3,'    CLOSE THE VALVE')
102    FORMAT(/5X,I5,8K,F10.3,5X,F10.3,F8.3,'    OPEN  THE VALVE')
      END
```

```
// FOR SCAN
      SUBROUTINE SCAN
       CALL LEVEL(11)
        RETURN
         END
*STORECI   M          PL2    GOEL   PL2
*CCEND
// JOB
// DUP
*CCEND
// JOB
// DUP
*DELETE    I          WS     WS1          2 11
// FOR WS
*ONE WORD INTEGERS
      COMMON/INSKEL/IPH
       DATAK/Z8000/
       CALL MASK(K,-1)
      CALL AIP(11000,IPH,10)
11    CALL AIP(0,ITEST)
       GOTO(11,12),ITEST
12    CONTINUE
       CALL INTEX
      END
*STORECI             WS1    WS
*CCEND
```

APPENDIX VIII

TABLE 5: ABSORBANCE AND SUGAR CONTENT OF DIFFERENT SAMPLES TAKEN DURING FERMENTATION

Sample No.	Time (Hrs)	Absorbance of the sample	Sugar concentration gm. ml^{-1} x 10^2	Per cent sugar concentration
1	0.0	0.82	5.9	5.9
2	1.0	0.59	2.9	2.9
3	1.416	0.53	2.2	2.2
4	2.086	0.50	1.8	1.8
5	2.670	0.47	1.5	1.5
6	3.170	0.44	1.2	1.2
7	3.670	0.42	0.95	0.95
8	4.254	0.40	0.75	0.75
9	4.67	0.38	0.55	0.55
10	4.75	0.368	0.37	0.37
11	5.169	0.360	0.27	0.27
12	5.669	0.35	0.19	0.19
13	6.169	0.34	0.12	0.12

APPENDIX VIII

TABLE 6: CALCULATIONS FOR DETERMINING:

(a) Amount of yeast present in the fermentor at different intervals o
(b) Product yield based upon substrate concentration time

Sample No.	Time (Hrs.)	Dry cell weight gms ml^{-1} (X)	Total amount of yeast in the fermentor gms (V=10 litres (P)	log P	Sugar concentration gms ml^{-1} (S)x 10^2	ΔX x 10^3	ΔS x 10^2	Product yield $Y_{x/s}=(\frac{\Delta X}{\Delta S})$
1	0.00	0.04060	406.0	2.60853	5.9	7.35	3.0	0.245
2	1.00	0.04795	479.5	2.68079	2.9	2.38	0.7	0.340
3	1.416	0.05033	503.3	2.70183	2.2	1.52	0.4	0.380
4	2.086	0.05185	518.5	2.71475	1.8	0.95	0.3	0.317
5	2.670	0.05280	528.0	2.72263	1.5	0.85	0.3	0.283
6	3.170	0.05375	537.5	2.73035	1.2	0.80	0.25	0.320
7	3.670	0.05465	546.5	2.73759	0.95	0.60	0.20	0.300
8	4.254	0.05525	552.5	2.74233	0.75	0.60	0.20	0.300
9	4.670	0.05585	558.5	2.74702	0.55	0.50	0.18	0.278
10	4.750	0.05635	563.5	2.75089	0.37	0.22	0.10	0.220
11	5.169	0.05657	565.7	2.75259	0.27	0.16	0.08	0.200
12	5.669	0.05673	567.3	2.75381	0.19	0.13	0.07	0.186
13	6.169	0.05686	568.6	2.75481	0.12			

APPENDIX IX

TABLE 7 : DATA LOGGING AND RESULTS FOR TEMPERATURE CONTROL

Time (in hours and millihours)	Volts	Temp.	Difference	Comment
13300	1.131	29.290	1.290	OPEN THE VALVE
13309	1.112	28.803	0.803	CLOSE THE VALVE
13318	1.110	28.751	0.751	CLOSE THE VALVE
13327	1.120	29.015	1.015	OPEN THE VALVE
13337	1.122	29.050	1.050	OPEN THE VALVE
13346	1.115	28.875	0.875	CLOSE THE VALVE
13355	1.108	28.685	0.685	CLOSE THE VALVE
13364	1.112	28.802	0.802	CLOSE THE VALVE
13374	1.120	29.015	1.015	OPEN THE VALVE
13383	1.191	30.786	2.786	OPEN THE VALVE
13392	1.141	29.534	1.534	OPEN THE VALVE
13401	1.060	27.520	-0.479	CLOSE THE VALVE
13411	1.047	27.184	-0.815	CLOSE THE VALVE
13420	1.091	28.283	0.283	CLOSE THE VALVE
13429	1.131	29.290	1.290	OPEN THE VALVE
13438	1.112	28.802	0.802	CLOSE THE VALVE
13448	1.103	28.588	0.588	CLOSE THE VALVE
13457	1.148	29.717	1.717	OPEN THE VALVE
13466	1.187	30.694	2.694	OPEN THE VALVE
13475	1.120	29.015	1.015	OPEN THE VALVE

Table (contd)

Time (in hours and millihours)	Volts	Temp.	Difference	Comment
13484	1.107	28.680	0.680	CLOSE THE VALVE
13494	1.091	28.283	0.283	CLOSE THE VALVE
13503	1.132	29.321	1.321	OPEN THE VALVE
13512	1.088	28.222	0.222	CLOSE THE VALVE
13521	1.048	27.215	-0.784	CLOSE THE VALVE
13531	1.082	28.052	0.052	CLOSE THE VALVE
13540	1.159	29.992	1.992	OPEN THE VALVE
13549	1.165	30.145	2.145	OPEN THE VALVE
13558	1.132	29.321	1.321	OPEN THE VALVE
13568	1.114	28.850	0.850	CLOSE THE VALVE
13577	1.125	29.125	1.125	OPEN THE VALVE
13586	1.112	28.802	0.802	CLOSE THE VALVE
13595	1.110	28.751	0.751	CLOSE THE VALVE
13605	1.113	28.825	0.825	CLOSE THE VALVE
13614	1.122	29.050	1.050	OPEN THE VALVE
13623	1.125	29.125	1.125	OPEN THE VALVE
13632	1.082	28.052	0.052	CLOSE THE VALVE
13642	1.051	27.275	-0.724	CLOSE THE VALVE
13651	1.091	28.283	0.283	CLOSE THE VALVE
13660	1.122	29.050	1.050	OPEN THE VALVE
13669	1.127	29.175	1.175	OPEN THE VALVE
13679	1.114	28.850	0.850	CLOSE THE VALVE

AND SO ON

APPENDIX IX

TABLE 8

DATA LOGGING AND RESULTS FOR pH CONTROL

Time (in hours and millihours)	Volts	pH	Difference	Comment
13015	1.864	4.653	0.146	OPEN THE VALVE
13018	1.872	4.705	0.094	CLOSE THE VALVE
13021	1.870	4.696	0.103	OPEN THE VALVE
13025	1.872	4.711	0.088	CLOSE THE VALVE
13029	1.872	4.707	0.092	CLOSE THE VALVE
13032	1.871	4.698	0.101	OPEN THE VALVE
13036	1.872	4.707	0.092	CLOSE THE VALVE
13040	1.875	4.727	0.072	CLOSE THE VALVE
13043	1.873	4.716	0.083	CLOSE THE VALVE
13047	1.875	4.729	0.070	CLOSE THE VALVE
13050	1.873	4.718	0.081	CLOSE THE VALVE
13055	1.870	4.693	0.106	OPEN THE VALVE
13058	1.870	4.696	0.103	OPEN THE VALVE
13062	1.871	4.700	0.099	CLOSE THE VALVE
13065	1.875	4.722	0.077	CLOSE THE VALVE
13069	1.870	4.693	0.106	OPEN THE VALVE
13073	1.871	4.700	0.099	CLOSE THE VALVE
13076	1.869	4.689	0.110	OPEN THE VALVE
13080	1.869	4.689	0.110	OPEN THE VALVE

Table (contd)

Time (in hours and millihours)	Volts	pH	Difference	Comment
13084	1.869	4.687	0.112	OPEN THE VALVE
13087	1.870	4.691	0.108	OPEN THE VALVE
13091	1.884	4.796	0.003	CLOSE THE VALVE
13095	1.871	4.698	0.101	OPEN THE VALVE
13099	1.872	4.707	0.092	CLOSE THE VALVE
13102	1.871	4.702	0.097	CLOSE THE VALVE
13105	1.873	4.714	0.085	CLOSE THEVALVE
13110	1.873	4.716	0.083	CLOSE THE VALVE
13113	1.872	4.705	0.094	CLOSE THEVALVE
13116	1.870	4.693	0.106	OPEN THE VALVE
13120	1.871	4.698	0.101	OPEN THE VALVE
13124	1.870	4.693	0.106	OPEN THE VALVE
13127	1.874	4.725	0.074	CLOSE THE VALVE
13131	1.872	4.705	0.094	CLOSE THE VALVE
13135	1.872	4.705	0.094	CLOSE THE VALVE
13139	1.869	4.687	0.112	OPEN THE VALVE
13142	1.871	4.702	0.097	CLOSE THE VALVE
13145	1.872	4.711	0.088	CLOSE THE VALVE
13150	1.872	4.707	0.092	CLOSE THE VALVE
13153	1.872	4.705	0.094	CLOSE THE VALVE
13156	1.872	4.711	0.088	CLOSE THE VALVE
13160	1.872	4.705	0.094	CLOSE THE VALVE
13164	1.872	4.705	0.094	CLOSE THE VALVE

Table (contd)

Time (in hours and millhours)	Volts	pH	Difference	Comment
13168	1.870	4.696	0.103	OPEN THE VALVE
13171	1.875	4.727	0.072	CLOSE THE VALVE
13175	1.872	4.705	0.094	CLOSE THE VALVE
13179	1.869	4.687	0.112	OPEN THE VALVE
13182	1.873	4.718	0.081	CLOSE THE VALVE
113186	1.873	4.718	0.081	CLOSE THE VALVE
13190	1.871	4.698	0.101	OPEN THE VALVE
13193	1.872	4.705	0.094	CLOSE THE VALVE
13197	1.872	4.707	0.092	CLOSE THE VALVE
13200	1.872	4.705	0.094	CLOSE THE VALVE
13205	1.871	4.698	0.101	OPEN THE VALVE
13208	1.871	4.700	0.099	CLOSE THE VALVE
13212	1.872	4.705	0.094	CLOSE THE VALVE
13215	1.871	4.698	0.101	OPEN THE VALVE
13220	1.875	4.729	0.070	CLOSE THE VALVE
13223	1.872	4.705	0.094	CLOSE THE VALVE
13226	1.869	4.687	0.112	OPEN THE VALVE
13230	1.868	4.682	0.117	OPEN THE VALVE
13234	1.871	4.700	0.099	CLOSE THE VALVE
13238	1.873	4.718	0.081	CLOSE THE VALVE
13241	1.869	4.687	0.112	OPEN THE VALVE

AND SO ON